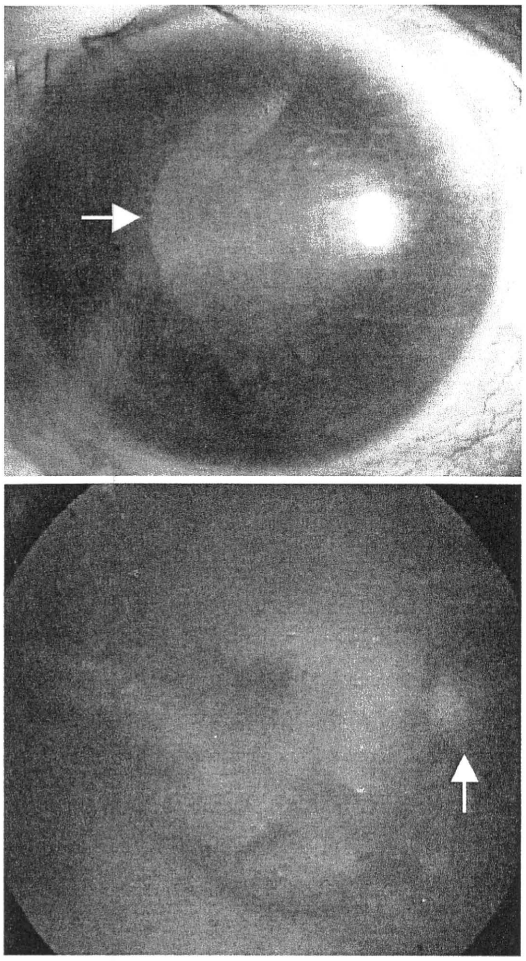

図 2　眼内検体を用いて PCR を施行した代表症例写真
スリット検査で，図のような麻痺性散瞳（矢印，上写真）があった．その他色素を含む豚脂様角膜後面沈着物，びまん性角膜浮腫，前房出血がみられていた．眼底は硝子体混濁のため視神経乳頭（矢印，下写真）がぼんやりと見えるほど透見不良であった．初診時の採取した前房水から VZV-DNA および EBV-DNA も同定され，抗ウイルス剤の内服と眼軟膏，ステロイド点眼でやがて消炎した．

産物を混合し，melting curve 解析を行い，ウイルスの検出を行った．これらは Tm 値（melting temperature，融解温度）が重ならないように設定したプローブによってウイルスの種類を判定した．

前房水のマルチプレックス PCR で，VZV（水痘・帯状ヘルペスウイルス）-DNA が検出された（図 1）．また同検体から EBV（Epstein-Barr ウイルス）-DNA も同定されていた（図 1）．その他 HSV1，HSV2，CMV，HHV6，HHV7，HHV8-DNA はすべて陰性であった．

4 リアルタイム定量 PCR 検査および経過

上記ウイルスのスクリーニング検査で VZV-DNA および EBV-DNA が陽性だったので，この 2 つのウイルスのリアルタイム定量 PCR 検査を行った．その結果，VZV-DNA が 1.1×10^8 copies/m*l*，EBV-DNA が 1.7×10^3 copies/m*l* といずれも高コピー数が検出されていた．特に，VZV のコピー数がきわめて高く VZV が主要ウイルスと考えられた．

この 2 つの PCR 検査結果より VZV 関連ぶどう膜炎と診断し，治療にバラシクロビル内服，ゾビラックス眼軟膏とデキサメタゾン点眼を用いた．3 週後にはほぼ消炎したが，内服中止可能かの判定のため再度前房水を採取し，リアルタイム定量 PCR を行った．両ウイルスとも DNA が検出限界以下（50 copies/m*l*）になっていたので，治療を中止した．その後，麻痺性散瞳と虹彩萎縮が残るも再発もなく経過良好である．

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32 ウイルス性虹彩毛様体炎

主な病原体
単純ヘルペスウイルス(herpes simplex virus：HSV)1型
水痘帯状疱疹ウイルス(varicella-zoster virus：VZV)
サイトメガロウイルス(cytomegalovirus：CMV)

ウイルス性虹彩毛様体炎とは

ウイルス性虹彩毛様体炎は、虹彩や毛様体に主座を有する眼内炎症性疾患で、主にヘルペスウイルスが原因で起こる。ヒトヘルペスウイルス科の多くは眼内組織に潜伏していて、何かを契機にウイルスが再活性化する。実際、眼内組織がウイルスレセプターをいくつか発現し、ヘルペスウイルスはその親和性が高いとされている。片眼性の高眼圧を伴う急性肉芽腫性虹彩毛様体炎ではまずこの疾患を疑う。

■ 主な臨床所見

ウイルス性虹彩毛様体炎の特徴的な眼所見として豚脂様角膜後面沈着物（**図1**）、高眼圧、角膜浮腫、虹彩萎縮、硝子体混濁などが挙げられ、基本的に片眼性に、急性にみられることが多い。ヘルペス性虹彩毛様体炎の場合、経過中に虹彩萎縮と麻痺性散瞳が認められるのが特徴的である（**図2**）。硝子体混濁は前部硝子体を中心にみられ、慢性化すると治療に反応せず、器質化して視力障害の原因となる。

■ 主な検査

診断には前房水を用いたウイルスPCRが最も信頼性が高く、迅速な診断が得られる[51]。上記の特徴的な眼所見と眼局所のウイルス遺伝子の同定で診断する。よく同定されるウイルスは、HSV-1、VZV、CMVである。血液中のウイルス抗体価はあまり有用ではないので参考程度にする。

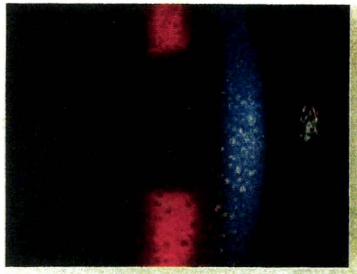

図1 HSV-1関連虹彩毛様体炎（豚脂様角膜後面沈着物）

図2 VZV関連虹彩毛様体炎（虹彩萎縮と麻痺性散瞳）

■ 鑑別診断

しばしばサルコイドーシスとの鑑別が困難であるが、このぶどう膜炎は両眼性に多く、特徴的な硝子体混濁や眼底の所見があり、年齢は中高年の女性が多い。

■ 治療

上記の特徴的な眼所見およびステロイド点眼投与でなかなか軽快しない片眼性の虹彩毛様体炎をみた時には、第一にウイルス性虹彩毛様体炎を疑う。治療には抗ウイルス薬内服（バラシクロビルやアシクロビル）が中心で、症状・所見に応じてステロイド点眼薬、眼圧下降薬を併用する。

（杉田　直）

Column Multiplex PCR

Polymerase chain reaction（PCR）とはポリメラーゼ連鎖反応のことで、遺伝子配列の決定や遺伝子の定量など、遺伝子研究の基本技術として確立されている。臨床の場では、ウイルス、細菌、真菌などの診断方法として応用され、眼科領域では現在、ぶどう膜炎、特にウイルス感染での確定診断のために PCR は有効な検査手段となっている。

近年、この PCR を応用して開発されたのが multiplex PCR（多項目迅速 PCR）検査である（**図1**）。この multiplex PCR 検査の最大の特徴は、数種類のウイルスなどの外来性抗原を同時に迅速に検出できる点にある。以前の一般的に行われていた PCR のようなゲル内のバンド検出で判定するのではなく、融解曲線で陽性か陰性かの判定を行う（**図2**）。曲線が大きい場合、DNA 量が多いことがわかり半定量できる利点がある。サンプル調整から PCR にかかる所要時間は2時間弱と従来の PCR の中でも迅速で、場合によっては10項目以上の外来性抗原 DNA の判定ができる。眼科領域での利点は、眼表面炎症性疾患（角膜炎、結膜炎など）の涙液検体は複数の外来性抗原が検出される可能性があり、この multiplex PCR は有用と思われる。また眼科では多くの症例で検体が微量であるので、この手法は極めて有用である。また近年の PCR システムの改良により、多種のウイルスなどの外来性抗原を同時にかつ迅速にこの PCR でスクリーニングして、その後異なったプライマーとプローブの組み合わせでウイルス量の定量化検査（real-time PCR）を行う検査システムが報告されている。その他の利点として、多くの感染性ぶどう膜炎や眼内炎を否定する目的で使用でき、治療の中心がステロイド薬のぶどう膜炎分野ではこれらの PCR が広く使用されている。

欠点としては、複数の PCR 反応を同時に進めるため、複雑なプライマーの設定などに技術と時間を要することが挙げられる。

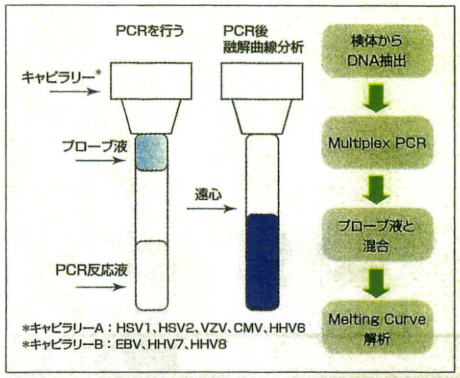

図1 Multiplex PCR 検査法
Multiplex PCR 検査は、melting Curve 解析（融解曲線分析）でウイルス遺伝子の同定を行う

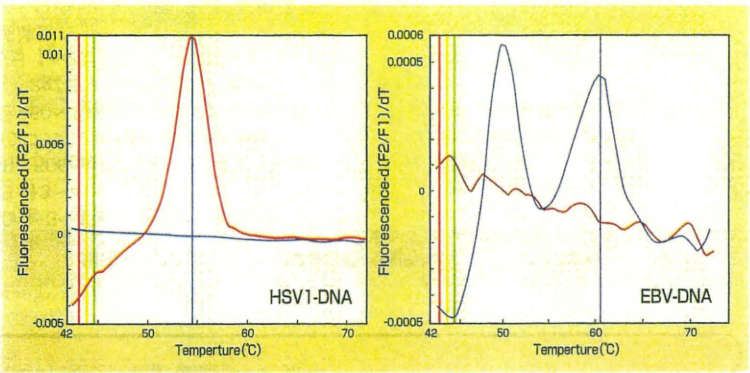

図2 Multiplex PCR の結果
ウイルス性虹彩毛様体炎患者の前房水を用いた multiplex PCR にて HSV-1 と EBV の2種類のウイルス DNA が検出された

（杉田　直）

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CLINICAL INVESTIGATION

Diagnosis of Intraocular Lymphoma by Polymerase Chain Reaction Analysis and Cytokine Profiling of the Vitreous Fluid

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Abstract

Purpose: To determine whether a diagnosis of intraocular lymphoma (IOL) can be made using a combination of polymerase chain reaction (PCR) analysis to detect gene rearrangement of immunoglobulin and cytokine concentrations in the vitreous fluid.

Methods: Vitreous samples from 22 patients with clinically suspected IOL and ten control patients with acute retinal necrosis or cytomegalovirus retinitis were examined by PCR analysis and cytokine measurements. Genomic DNA was extracted from the cells in the vitreous, and the immunoglobulin heavy chain (*IgH*) gene was amplified by two PCR procedures: (1) microdissection and PCR to detect *IgH* gene rearrangement and (2) qualitative PCR to detect *IgH* VDJ gene rearrangement. The supernatants of the vitreous samples were used for enzyme-linked immunosorbent assay to determine interleukin (IL)-10 and IL-6 levels.

Results: PCR examinations detected *IgH* rearrangement in the vitreous in 21 of the 22 IOL patients (95.5%) and in none of the ten control patients. Elevated IL-10 concentrations (>100 pg/ml) and the IL-10/IL-6 ratio (>1.0) were positive in 18 of the 22 IOL patients (81.8%), but negative in all of the control patients. Sensitivity, specificity, positive predictive value, and negative predictive value of PCR for the diagnosis of IOL were calculated to be 0.955, 1.000, 1.000, and 0.909, respectively, and those of the cytokine concentration assay to be 0.818, 1.000, 1.000, and 0.714, respectively. When both the intravitreal cytokine assay and PCR analysis of the vitreous samples are used, as well as diagnostic criteria of IOL defined as a positive outcome from one of the two assays together with clinical signs, the sensitivity and specificity of the criteria were 1.000.

Conclusions: A combination of PCR assay to detect gene rearrangement of *IgH* and cytokine profiling (IL-10 and IL-6) is extremely useful for the diagnosis of intraocular lymphoma. **Jpn J Ophthalmol** 2009;53:209–214 © Japanese Ophthalmological Society 2009

Key Words: IL-10, immunoglobulin gene rearrangement, intraocular lymphoma, polymerase chain reaction, vitreous fluid

Introduction

Intraocular lymphoma (IOL) is a high-grade malignant non-Hodgkin lymphoma most often of a B-cell type that affects the retina, choroid, vitreous, or the optic nerve.[1-3] IOL is considered to be a subtype of primary central nervous system lymphoma (CNS lymphoma). When IOL is diagnosed and no CNS lesions are detected, the condition is referred to as primary IOL. In addition, IOL can occur as secondary lesions following systemic lymphoma and is then referred to as metastatic IOL. Histopathologically, most IOLs are classified according to the World Health Organization Classification for Hematologic and Lymphoid Neoplasms as diffuse large B-cell lymphomas.

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Clinically, IOL is commonly found in elderly patients with a nonspecific corticosteroid-resistant uveitis. At the initial presentation, IOL exhibits signs and symptoms very similar to uveitis and is often treated with corticosteroids. Diagnosis of IOL cannot be made on the basis of clinical signs and symptoms. Furthermore, cytological diagnosis using vitreous samples is often negative, in part because of poor biopsy samples. Polymerase chain reaction (PCR) has been used examine monoclonal rearrangements of immunoglobulin heavy chain (*IgH*) in B-cell lymphoma,[4–6] and the interleukin (IL)-10 concentration[7,8] and the IL-10/IL-6 ratio[8] in ocular fluids have also been used. Although many reports[9–11] support the value of these diagnostic investigations, some studies have reported false-positive or false-negative results from these diagnostic tests for IOL.[12]

The aim of the present study therefore was to determine whether a combination of these two investigational tests of vitreous biopsy samples could be used for the diagnosis of IOL.

Materials and Methods

Subjects

Vitreous fluid samples from 22 patients with clinically suspected IOL and ten controls with acute retinal necrosis (ARN) (seven patients) or cytomegalovirus retinitis (three patients) were examined between 2000 and 2007 at Tokyo Medical and Dental University Hospital. IOL was clinically suspected in a patient when either vitreous opacities or subretinal white lesions with unknown etiology or with systemic lymphoma/CNS lymphoma were observed, and when all routine diagnostic laboratory tests for uveitis were negative. As for the ten control patients, all had active intraocular inflammation at the time of sampling. An aliquot (0.8–1.0 ml) of vitreous fluids was collected at the beginning of the vitrectomy.

Informed consent was obtained from each patient before the sample collection. The research followed the tenets of the Declaration of Helsinki, and the Institutional Ethics Committees of Tokyo Medical and Dental University approved all study protocols.

Cytological Examination

Specimens were centrifuged at 7280 *g* for 8 min at 4°C. The cell pellet was then resuspended in fresh RPMI-1640 medium, cytocentrifuged for 10 min, and placed directly onto a gelatinized slide. Giemsa- and Gram-stained slides were prepared and examined at the Pathology Department of our hospital. The results of the cytological examination were classified according to Papanicolaou classes I–V.

Polymerase Chain Reaction for IgH *Gene Rearrangement*

Immunoglobulin heavy chain (*IgH*) gene rearrangement of B-cell lymphoma in the vitreous fluids was examined using two independent PCR assays: (1) gene rearrangement by microdissection and PCR and (2) *IgH* VDJ gene rearrangement by qualitative PCR.

Microdissection was performed by Chi-Chao Chan at the Laboratory of Immunology of the National Eye Institute (NEI) in the United S tates as follows. In brief, atypical cells with large nuclei were identified under a light microscope in paraffin sections stained with H&E. The histologic area of interest on the slide was gently scraped with a 30-gauge needle until the selected cells were detached from the tissue section. DNA was extracted from the microdissected cells, and PCR was performed as previously described.[4,5,13] The search used primers for the third framework (FR3A), the second framework (FR2A), and the complementary determining region 3 (CDR3) of the V_H region. Independently, we also performed a conventional qualitative PCR for diagnosis of IOL at our laboratory. The primers for the *IgH* VDJ gene and the PCR conditions, described previously,[14] differed from those used at NEI. The products were subjected to PCR in the presence of the primers (1 pmol), 200 mM deoxynucleotide triphosphates, and 2.5 U of *Taq* polymerase in a buffer containing 50 mM KCl and 1.5 mM $MgCl_2$. Samples were subjected to 40 cycles of amplification consisting of denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and polymerization for 1 min at 72°C. As a positive control, Raji cell lines (Burkitt lymphoma cells) were used. The PCR products were analyzed by 10% polyacrylamide gel electrophoresis and ethidium bromide staining. All vitreous samples were tested for the presence of β-actin as an internal control.

The PCR results for *IgH* gene rearrangement were taken as supportive of the diagnosis of IOL when either microdissection and PCR at NEI (FR3A, FR2A, or CDR3 gene) or PCR at our laboratory (VDJ gene) was positive.

Enzyme-Linked Immunosorbent Assay for Measurement of IL-10 and IL-6 in the Vitreous

Using the supernatants of the vitreous samples, we determined the concentrations of IL-6 and IL-10 using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA). A minimum of 50 µl of each diluted vitreous sample was used for the ELISA assay. Samples were stored at –80°C until use, and cytokine assays were performed on freshly thawed samples.

The cytokine assay of the vitreous fluids of the IOL patients was taken as supportive of the diagnosis of IOL when IL-10 concentrations were >100 pg/ml and the IL-10/IL-6 ratio was >1.0.

Diagnostic Values of IgH *Gene Rearrangement and Cytokine Assay of the Vitreous Samples*

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the detection of *IgH* gene rearrangement by PCR and of the cytokine assay of the vitreous samples were calculated from the clinical data.

Statistical Analysis

Statistical analysis was performed using the Mann-Whitney *U* test. Statistical significance was set at $P < 0.05$.

Results

Clinical Features

The clinical characteristics of the patients are summarized in Table 1. All patients had clinically suspected IOL with retinal or vitreous lesions, and in all patients uveitis was clinically excluded. Of the 22 IOL patients, 13 had primary IOL limited to the eyes at the time of diagnosis and no evidence of CNS, systemic, or spinal cord lymphoma; two had CNS lymphoma (cases 14 and 15); seven had metastatic IOL with lymphoma in organs other than the CNS. All metastatic IOL patients and CNS lymphoma patients had been previously diagnosed as having lymphoma in the respective organs when they came to our clinic.

Cytological Examination of Patients with IOL

Almost all of the vitreous samples of patients with suspected IOL were submitted to the Pathology Unit of our hospital for cytological examination. Of the 20 IOL patients, only four (20%) were histopathologically diagnosed as having lymphoma (class V); most were classified as class II or III (Table 2). The details of the diagnostic examination based on the vitreous samples are summarized in Table 2. Most samples were defined as class III (19/28, 68%), with four samples defined as class II (14%), and the one remaining sample as class IV (only 4%). In one patient (case 17 in Table 2), no cells were detected in the samples. Although case 7 was classified as primary IOL, the patient did not undergo cytological examination at the first evaluation because the patient had been clinically diagnosed as having ARN.

IgH *Gene Rearrangement Detected by PCR of the Vitreous Samples*

PCR to detect *IgH* gene rearrangement was carried out at either NEI or our laboratory in Tokyo in 22 patients with suspected IOL and ten control patients (Table 2). *IgH* gene rearrangements (VDJ, FR3A, FR2A, or CDR3) were detected in 21 of the 22 patients with suspected IOL (95.5%). In only one patient (case 8 in Table 2) was *IgH* gene rearrangement negative according to the PCR assays at both NEI and our laboratory. On the other hand, in none of the ten patients with ARN or cytomega-

Table 1. Intraocular lymphoma clinical details

Case	Age (years)	Sex	Primary organ	Diagnosis	Ocular lesions at initial presentation	CNS involvement	Current status
1	69	F	Eye	Primary IOL	Vitreous & retina	Yes	Deceased
2	79	F	Eye	Primary IOL	Vitreous	No	Alive
3	62	F	Eye	Primary IOL	Vitreous & retina	Yes	Deceased
4	76	F	Eye	Primary IOL	Vitreous & retina	No	Alive
5	57	M	Eye	Primary IOL	Vitreous & retina	Yes	Deceased
6	70	M	Eye	Primary IOL	Retina	No	Alive
7	56	M	Eye	Primary IOL	Vitreous & retina	Unknown	Unknown
8	82	F	Eye	Primary IOL	Retina	No	Alive
9	78	M	Eye	Primary IOL	Vitreous & retina	No	Alive
10	73	F	Eye	Primary IOL	Vitreous	No	Alive
11	75	F	Eye	Primary IOL	Retina	No	Alive
12	56	F	Eye	Primary IOL	Retina	Yes	Deceased
13	70	M	Eye	Primary IOL	Vitreous	No	Alive
14	69	M	Brain (CNS)	Primary IOL	Vitreous & retina	Yes	Deceased
15	66	M	Brain (CNS)	Primary IOL	Vitreous	Yes	Alive
16	82	F	Pharynx	Metastatic IOL	Vitreous	No	Alive
17	86	F	Neck	Metastatic IOL	Vitreous & retina	Yes	Deceased
18	45	M	Appendix	Metastatic IOL	Vitreous	No	Alive
19	67	M	Stomach	Metastatic IOL	Vitreous	No	Alive
20	38	F	Breast	Metastatic IOL	Vitreous	Yes	Deceased
21	87	M	Head	Metastatic IOL	Vitreous & retina	Yes	Deceased
22	73	M	Testis	Metastatic IOL	Retina	No	Alive

Patients 1 to 15 had primary IOL, including CNS lymphoma. Patients 16 to 22 had metastatic IOL.
IOL, intraocular lymphoma; CNS, central nervous system.

Table 2. Vitreous fluid examinations of patients with intraocular lymphoma and in controls

Case	Diagnosis	Source	Cytology (R/L)	ELISA (R/L) IL-10 (pg/ml)	ELISA (R/L) IL-6 (pg/ml)	IL-10/IL-6 ratio	PCR (R/L) Microdissection & PCR	PCR (R/L) *IgH* VDJ rearrangement
1	Primary IOL	VF	III	5000	50	100	(+)	nd
2	Primary IOL	VF	III	<10	<10	–	(+)	(−)
3	Primary IOL	VF	III	1075	20	54	(+)	(+)
4	Primary IOL	VF	IIIb	15000	70	214	(+)	(+)
5	Primary IOL	VF	II / V	24080 / 43479	11298 / 1245	2 / 35	nd / nd	(+) / (+)
6	Primary IOL	VF	II	33	61	0.5	nd	(+)
7	Primary IOL	VF	nt	2364	387	6	nt	(+)
8	Primary IOL	VF	III	1233	148	8	nt	nd
9	Primary IOL	VF	III / V	1923 / 138	37 / <10	52 / 14	nt / nt	(+) / nt
10	Primary IOL	VF	IV / III	109 / 172	31 / 30	4 / 6	(−)	(−) / (+)
11	Primary IOL	VF	V / III	3395 / 238	1891 / 144	2 / 2	nt / nt	(+) / (+)
12	Primary IOL	VF	III	250	50	5	(−)	(+)
13	Primary IOL	VF	III	348	5	70	nt	(+)
14	Primary IOL	VF	III / II	82 / <10	<10 / <10	8 / –	nt	(+) / (−)
15	Primary IOL	VF	V	4643	157	30	nt	(+)
16	Metastatic IOL	VF	IIIb	2053	12	171	nd	(+)
17	Metastatic IOL	VF	nd	16000	1050	15	(+)	nt
18	Metastatic IOL	VF	III	1680	119	14	nt	(+)
19	Metastatic IOL	VF	III / III	126 / 207	201 / 113	0.6 / 1.8	(−) / nt	(+) / (+)
20	Metastatic IOL	VF	IIIb / III	470 / 64	15 / 46	31 / 1.4	(+) / (−)	(−) / (+)
21	Metastatic IOL	VF	III / IIIb	380 / 580	250 / 350	1.5 / 1.7	(+) / (−)	(+) / (+)
22	Metastatic IOL	VF	II	505	733	0.7	(−)	(+)
23	ARN	VF	nt	90	1080	0.1	nt	(−)
24	ARN	VF	nt	764	956	0.8	nt	(−)
25	ARN	VF	nt	172	22759	0.01	nt	(−)
26	ARN	VF	nt	122	660	0.2	nt	(−)
27	ARN	VF	nt	375	24404	0.02	nt	(−)
28	ARN	VF	nt	201	1833	0.1	nt	(−)
29	ARN	VF	nt	188	834	0.2	nt	(−)
30	CMVR	VF	nt	<10	874	0	nt	(−)
31	CMVR	VF	II	<10	12929	0	nt	nt
32	CMVR	VF	nt	<10	724	0	nt	nt

The cytology results were classified according to the Papanicolaou classes I–V. Microdissection and PCR detected the *IgH* rearrangement gene in FR3A, FR2A, and CDR3.

PCR for B-cell lymphoma was also performed using specific primers for *IgH* VDJ rearrangement.

ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; IL, interleukin; nd, no decision (due to lack of sufficient sample volume, insufficient cell number, or negative β-actin internal control); nt, not tested (due to limited tissue); ARN, acute retinal necrosis; CMVR, cytomegalovirus retinitis; VF, vitreous fluid; ARN, acute retinal necrosis.

lovirus retinitis, was *IgH* gene rearrangement detected by PCR.

IL-10 and IL-6 Concentrations in the Vitreous Samples

The intravitreal concentrations of IL-10 and IL-6 in the IOL patients and the control patients with ARN and cytomegalovirus retinitis are summarized in Table 2. The mean concentrations of IL-10 and IL-6 in the 30 vitreous samples of the 22 IOL patients were 4187 ± 1696 pg/ml and 617 ± 377 pg/ml, respectively. On the other hand, the mean concentrations of IL-10 and IL-6 in the ten vitreous samples of the ten control patients were 181 ± 75 pg/ml and 6705 ± 3053 pg/ml, respectively. In the patients with IOL, the IL-10 concentrations were significantly higher than those of IL-6 ($P = 0.0006$), whereas in the control patients, the IL-6 concentrations were significantly higher than those of IL-10 ($P = 0.0003$). The IL-10/IL-6 ratio was >1.0 in all but three of the patients with IOL (cases 2, 6, and 22), whereas it was <1.0 in all of the control patients.

Eighteen of the 22 (81.8%) patients with suspected IOL met the criteria of a cytokine profile supportive of the diagnosis of IOL, that is, IL-10 concentration >100 pg/ml and IL-10/IL-6 >1.0. On the other hand, none of the ten control patients met the criteria.

Diagnostic Parameters of IgH *Gene Rearrangement and Cytokine Profiling of the Vitreous*

The diagnostic parameters of sensitivity, specificity, PPV, and NPV of the molecular examinations (*IgH* gene rearrangement by PCR) for the diagnosis of IOL were calculated to be 0.955, 1.000, 1.000, and 0.909, respectively. The

sensitivity, specificity, PPV, and NPV of the immunological tests (IL-10 >100 pg/ml and IL-10/IL-6 >1.0) in the vitreous were calculated to be 0.818, 1.000, 1.000, and 0.714, respectively. If we define the diagnostic criteria for IOL as a positive outcome in one of the two assays (intravitreal cytokine assay and PCR) together with clinical signs, all of our 22 patients with suspected IOL could be diagnosed as having IOL and all ten control patients as not having IOL, indicating that the sensitivity and specificity of the criteria were 1.000.

Discussion

The aim of the present study was to determine whether a molecular examination detecting the *IgH* gene rearrangement by PCR and an immunological test measuring the IL-10 and IL-6 concentrations in the vitreous fluid provide supportive evidence for the diagnosis of IOL. The data clearly showed that both the molecular examination and the immunological test were diagnostic of IOL, as demonstrated by the high values of sensitivity, specificity, PPV, and NPV of each investigation and of the combination of the two. In addition, positive criteria from either the molecular examination or the immunological test gave extremely high values for the diagnostic parameters.

In the present study, we used two independent PCR methods to detect *IgH* gene rearrangement: (1) microdissection and PCR to detect the FR3A, FR2A, or CDR3 genes and (2) conventional PCR to detect the VDJ gene. Because the primers and probes used in the two methods differed, we speculated that use of the two molecular investigations would increase the possibility of detecting *IgH* gene rearrangement than would use of a single PCR assay. A discrepancy was found in the PCR results between the two assays in some patients with suspected IOL, which was due in part to the differences in the methods used. However, a combination of the two PCR assays was useful in the diagnosis of IOL. *IgH* gene rearrangement was not detected by either PCR method in only one patient (case 8), but the patient had a high concentration of IL-10 (1233 pg/ml) and a low concentration of IL-6 (148 pg/ml), and an IL-10/IL-6 ratio of 8. In addition, this patient had clinically suspected IOL with multiple retinal exudates, and uveitis was clinically excluded.

With regard to the immunological testing of the vitreous fluid, all but one of the patients with suspected IOL (case 2) had detectable levels of IL-10 in the vitreous. In most of the patients with suspected IOL, the IL-10 concentration in the vitreous was very high, but in two patients (cases 6 and 14), it was <100 pg/ml. In three of these patients (cases 2, 6, and 14), the molecular investigation yielded positive results in that *IgH* gene rearrangement was detected by PCR. The IL-10/IL-6 ratio was >1.0 in all but three of the patients with suspected IOL (cases 2, 6, and 16). In the three patients with IL-10/IL-6 <1.0, the molecular test was also positive. In the control group, IL-10 was detectable in the vitreous in many patients, especially in all seven of the patients with ARN. However, the concentration of IL-6 in the vitreous was much higher than that of IL-10 in all of the IOL patients, and the IL-10/IL-6 ratio was <1.0 in all control patients. Ongkosuwito et al.[15] previously reported the cytokine profiles in the ocular fluids of 44 eyes with infectious uveitis. They found increased IL-6 levels in 44 control eyes and 43 eyes with infectious uveitis. Moreover, they detected IL-10 in ten eyes with ARN and 13 eyes with toxoplasmosis, but in only three control samples. As reported by many investigators,[7,8,11] primary IOL is strongly associated with an IL-10/IL-6 ratio >1.0.

The present molecular and immunological data suggest the following: (1) *IgH* gene rearrangement in vitreous cells can be negative in some IOL patients; (2) low or undetectable levels of IL-10 in the vitreous or an IL-10/IL-6 ratio <1.0 can be detected in some IOL patients; and (3) high IL-10 concentrations in the vitreous can be detected in some uveitis patients such as ARN patients. Thus, the data in the present study, together with those of many previous studies,[10,12,15,16] indicate that one cannot make a definite diagnosis of IOL on the basis solely of molecular investigations or solely of immunological tests of vitreous samples. In this study, therefore, we analyzed the values of four diagnostic parameters for the molecular investigation, the immunological test, and the combination of the two. We used the following criteria as results supportive of the diagnosis of IOL: (1) detection of *IgH* gene rearrangement by either of two PCR assays in the molecular investigation and (2) IL-10 >100 pg/ml or IL-10/IL-6 >1.0 in the immunological test. Although the sensitivity, specificity, PPV, and NPV of the molecular investigation alone were 0.955, 1.000, 1.000, and 0.909, respectively, and those of the immunological test alone were 0.818, 1.000, 1.000 and 0.714, respectively, and considered to be high, a positive outcome from one of two assays (IgH gene rearrangement by PCR and cytokine assay of the vitreous sample) gave much higher values for the diagnostic parameters: 1.000 for all four parameters. In the clinical situation, we need to make difficult decisions based on the diagnosis as to whether patients with suspected IOL should be treated with intraocular and systemic chemotherapy, which are very invasive. Therefore, the diagnosis should be as accurate as possible.

In conclusion, on the basis of a molecular investigation to detect *IgH* gene rearrangement and immunological testing to measure IL-10 and IL-6 in the vitreous, a patient who has the intraocular signs of suspected IOL and positive results from the molecular investigation and from the immunological testing of the vitreous sample must be considered as having IOL, and the treatment strategies should be decided accordingly.

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A significant association of viral loads with corneal endothelial cell damage in cytomegalovirus anterior uveitis

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A significant association of viral loads with corneal endothelial cell damage in cytomegalovirus anterior uveitis

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ABSTRACT

Aim The aim of the study was to investigate the correlation between the clinical manifestation and the cytomegalovirus (CMV) viral load in the aqueous humour of patients with CMV anterior uveitis.

Methods Seven patients with CMV-associated iridocyclitis and four patients with CMV-associated corneal endotheliitis were enrolled. Presence of CMV, but not other human herpes viruses, was confirmed by multiplex polymerase chain reaction (PCR). Viral load was measured using real-time PCR. Clinical manifestations were examined using a slit-lamp microscope and ophthalmoscope, applanation tonometer and specular microscope.

Results All 11 patients had unilateral recurrent anterior uveitis with high intraocular pressure and mutton fat keratic precipitates with pigmentation. Stromal oedema of the cornea was found in CMV-associated endotheliitis, but not in CMV-associated iridocyclitis patients. A significant corneal endothelium cell loss was recorded in all 11 patients with CMV-associated endotheliitis and iridocyclitis patients. High viral loads of CMV were detected in the aqueous humour of all 11 patients. A significant association was found between the corneal endothelial cell loss intensity and CMV viral load in the aqueous humour.

Conclusion There is a significant correlation between the CMV viral load and corneal endothelial cell loss in both CMV-associated iridocyclitis and corneal endotheliitis.

INTRODUCTION

Cytomegalovirus (CMV) is a member of the human herpes virus family and is found in latent infections in the majority of the adult population. In immunocompromised hosts, the virus causes necrotising retinitis,[1] but has been thought not to cause any diseases in immunocompetent hosts. However, a previous study showed local production of anti-CMV antibodies in the aqueous humour of an immunocompetent patient with iridocyclitis with elevated intraocular pressure (IOP).[2] In addition, recent studies using qualitative PCR have demonstrated that genomic CMV DNA is present in the aqueous humour of immunocompetent patients with unilateral iridocyclitis[3–6] as follows. Markomichelakis *et al*[3] reported two cases of iridocyclitis with sectoral iris atrophy in which CMV was detected by PCR, and de Schryver *et al*[4] also reported five similar cases. In the recent report by Chee *et al*,[5] they studied if there was a relationship between the CMV viral load in the aqueous and clinical manifestation of the diseases such as either acute or chronic iridocyclitis, eg Posner–Schlossman syndrome and Fuchs heterochromic iridocyclitis. CMV genomic DNA was also detected in the aqueous humour of immunocompetent patients with another inflammatory condition of the eye, ie corneal endotheliitis, in three previous reports.[7–9] Corneal endotheliitis is an inflammatory condition at the corneal endothelium in which keratic precipitates (KPs) develop together with severe stromal oedema in the cornea, whereas iridocyclitis has cells and flare in the anterior chamber with or without KPs but no stromal oedema in the cornea.

The real-time PCR made it possible to measure the viral load quantitatively. Thus, the use of this assay makes it possible to determine the clinical significance of the viral infection in the pathogenesis of human diseases. Our previous report showed a high CMV genomic DNA load in the aqueous humour in an immunocompetent patient with unilateral iridocyclitis with high IOP.[6] However, the correlation between the viral load in the aqueous humour and the clinical manifestation of the disease (iridocyclitis versus corneal endotheliitis) was not investigated. Therefore, we examined if there was any correlation between the CMV viral load in the aqueous humour and the clinical manifestation of anterior inflammatory diseases associated with CMV. We showed a significant correlation between the CMV viral load in the aqueous humour and the endothelial cell damage of the cornea in patients with iridocyclitis and corneal endotheliitis associated with CMV.

MATERIALS AND METHODS

Subjects

Between 2006 and 2008, 11 patients with CMV-associated inflammation in the anterior segment of the eye, ie seven patients with CMV-associated iridocyclitis and four patients with CMV-associated corneal endotheliitis, were enrolled. These patients were from Tokyo Medical and Dental University Hospital (Tokyo, Japan), Miyata Eye Hospital (Miyakonojo, Miyazaki, Japan) and Kyoto Prefectural University Hospital (Kyoto, Japan). Diagnosis was made based on clinical manifestations and the qualitative detection of the CMV genomic DNA in the aqueous humour by the multiplex PCR. The viral load in the aqueous humour was further measured quantitatively by the real-time PCR.

An aliquot of 0.1 ml of the aqueous humour was aspirated with a 30G needle after disinfection and

processed for PCR. Anti-viral therapy was not given before the PCR assay, but topical corticosteroids were given by local ophthalmologists to treat intense anterior uveitis. The interval between the disease onset and the aqueous humour sampling varied among the patients.

Polymerase chain reaction

The aqueous humour samples were centrifuged at 1000 *g* for 5 min and used for multiplex PCR and real-time PCR.[10 11] Multiplex PCR was designed to qualitatively measure the genomic DNA of eight human herpes viruses: herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella zoster virus (VZV), Epstein–Barr virus (EBV), CMV, and human herpes virus type 6 (HHV-6), type 7 (HHV-7) and type 8 (HHV-8). DNA was extracted from the aqueous humour samples using a DNA minikit (Qiagen, Valencia, California, USA). Multiplex PCR was performed using LightCycler (Roche, Basle, Switzerland). The primers of the glycoprotein gene sequences for CMV were TACCCCTATCGCGTG TGTTC (forward) and ATAG-GAGGCGCCACGTATTC (reverse). The probes used included 3′-fluorescein isothiocyanate: TCGTCGTAGCTACGCTTACAT and LcRed705-5′: ACACCACTTATCTGCTGGGCAGC. Specific primers for the virus were used in conjunction with Accuprim Taq (Invitrogen, Carlsbad, California, USA). PCR amplification conditions used in the current study have been reported previously.[12]

Real-time PCR was only performed for the HHV, with multiplex PCR used to detect the genomic DNA. Amplitaq Gold, with a Real-Time PCR 7300 system (ABI, Foster City, California, USA), was used to perform the procedure. The forward and reverse primers of immediate early (IE)-1 were CATGAAGGT-CTTTGCCCAGTAC and GGCCAAAGTGTAGGCTACAATAG, respectively. FAM-TGGCCCGTAGGTCATCCACACTAGG-TAMRA was used as the probe. The PCR amplification conditions used in the current study were previously reported by Sugita *et al*.[11] When more than 50 copies per tube (5×10^3/ml) were observed, the value of the sample's viral copy number was considered to be significant.

Clinical evaluation

Clinical manifestations of the eye were determined by a slit-lamp microscopic and ophthalmoscopic examination. Each patient underwent best corrected visual acuity (BCVA) measurement using a Japanese standard decimal visual acuity chart (Landolt ring chart) after treatment. Anterior chamber flare was measured by a laser flare photometer (FC-1000; Kowa Electronics, Nagoya, Japan). A photograph of the central cornea using a specular microscope (NONCON ROBO FA-3509; Konan Medical, Nishinomiya, Japan) was used for evaluation of the corneal endothelial cells. In cases of corneal endotheliitis, intense corneal oedema disturbed the measurements of the corneal endothelium, and we measured corneal endothelial cell counts after the inflammation was reduced by the treatment.

Evaluation of corneal endothelial cell loss

The relationship between the CMV viral load in the aqueous humour and the intensity of the corneal endothelial cell loss was assessed. The corneal endothelial cell loss was determined according to the following formula:

$$\text{Corneal endothelial cell loss}(\%) = 100 - (\text{endothelial cell counts in affected eye})/(\text{endothelial cell counts in the fellow eye})\times 100$$

Statistical analysis

Statistical analysis was performed using the Mann–Whitney U test. Statistical significance was set at $p<0.05$. Linear regression analysis was performed using the Spearman's correlation coefficient by rank test.

RESULTS

Clinical manifestations

Nine men and two women ranging in age from 23 to 71 years (mean age 60.6 years) were enrolled in the study. No abnormalities were found in the systemic investigations and laboratory tests. Serology examinations for human immunodeficiency virus were all negative. None of the patients had any history of eye surgery prior to the onset of uveitis. Clinical findings of the CMV-associated iridocyclitis patients (n=7) and corneal endotheliitis patients (n=4) are shown in table 1. A unilateral mild anterior uveitis with high IOP was noted in all 11 patients. There were no significant differences between the iridocyclitis and corneal endotheliitis groups in the cells and flare values in the anterior chamber, nor were there any differences noted for the elevated levels of IOP, KPs, gonioscopic findings and iris atrophy. Stromal oedema of the cornea was seen in all corneal endotheliitis but not in iridocyclitis patients. While the stromal oedema was diffuse in three out of the four patients, it was localised at upper cornea in one of the corneal endotheliitis patients. Representative cases for iridocyclitis and corneal endotheliitis are shown in figures 1 and 2, respectively. As for the IOP elevation, all 11 eyes required anti-glaucoma medications, with two eyes (cases 1 and 2) requiring trabeculectomy. With regard to the iris atrophy, no sectorial iris atrophy was seen in all 11 eyes, although four eyes (two each in the iridocyclitis and the corneal endotheliitis groups, respectively) presented diffuse iris atrophy.

Systemic valganciclovir therapy (1800 mg/day for longer than 3 weeks) in conjunction with topical corticosteroids and

Table 1 Clinical findings in patients with CMV anterior uveitis

Case	Age (years)	Sex	Eye	Diagnosis	Corneal oedema	KPs	Cells in AC	Flare in AC	IOP (mmHg)	Pigmentation in the AC angle	Iris atrophy
1	66	M	R	Iridocyclitis	-	Mutton-fat	1+	17	38	Depigmentation	None
2	62	M	R	Iridocyclitis	-	Mutton-fat	1+	26	40	PAS and pigment	Diffuse
3	56	M	L	Iridocyclitis	-	Mutton-fat	1+	13	44	Depigmentation	Diffuse
4	53	F	R	Iridocyclitis	-	Mutton-fat	1+	13	36	Depigmentation	None
5	71	M	L	Iridocyclitis	-	Mutton-fat	2+	28	25	PAS	None
6	63	M	R	Iridocyclitis	-	Fine	1+	Nt	50	Depigmentation	None
7	23	M	R	Iridocyclitis	-	Fine	1+	Nt	25	Depigmentation	None
8	71	M	R	Endotheliitis	+ (diffuse)	Mutton-fat	2+	151	37	PAS	None
9	67	M	R	Endotheliitis	+ (diffuse)	Fine	1+	14	25	Depigmentation	Diffuse
10	64	F	L	Endotheliitis	+ (superior)	Fine	1+	21	28	Depigmentation	None
11	71	M	R	Endotheliitis	+ (diffuse)	Mutton-fat	1+	12	43	PAS	Diffuse

Information from 11 patients with CMV anterior uveitis were reviewed. Data collected included intraocular pressure and clinical manifestation of the anterior segments in the affected eye.
AC, anterior chamber; F, female; KP, keratic precipitate; M, male; Nt, not tested; PAS, peripheral anterior synechia.

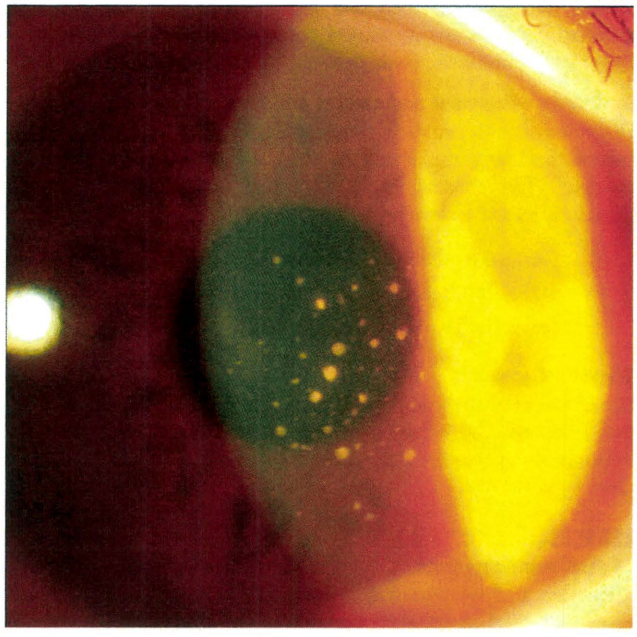

Figure 1 Case 4: Slit-lamp microscopy photo with cytomegalovirus-associated iridocyclitis. Mutton fat keratic precipitates with some pigmentation were scattered within the central area of the cornea. There was mild inflammation found within the anterior chamber.

anti-glaucoma agents effectively controlled the inflammation in the anterior segment of the eye as well as the high IOP.

Corneal endothelial cell loss

Specular microscopic examination revealed significant corneal endothelial cell loss (≥35%) in all 11 patients (table 2). Severe corneal endothelial cell loss larger than 70% was recorded in more than one-half of the endotheliitis group eyes. In contrast, this severe cell loss was observed in one of the seven patients with iridocyclitis.

There were several patients (cases 1, 8, 10 and 11; see table 2) with corneal endothelial cell counts <700 cells/mm^2. Among the patients, three cases had a low visual acuity between 0.3 and 0.6. However, one patient had a good visual acuity of 1.5.

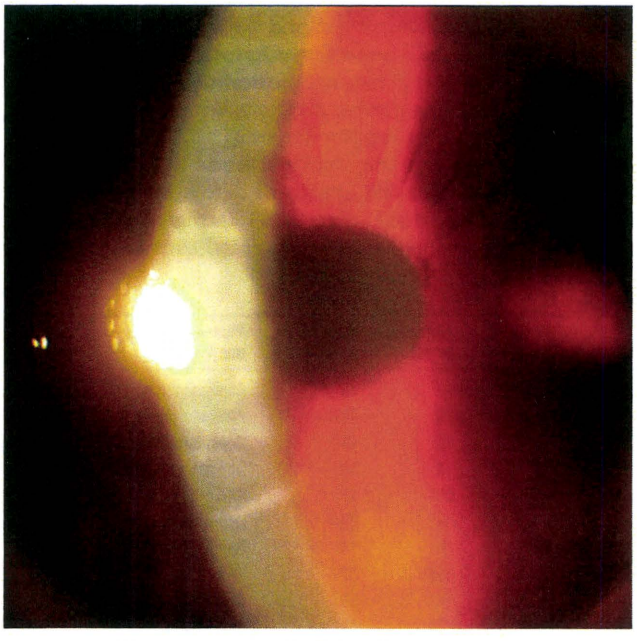

Figure 2 Case 8: Slit-lamp microscopy photo with cytomegalovirus-associated corneal endotheliitis. Diffuse corneal stromal oedema with folds in Descemet's membrane was observed.

PCR analysis of the aqueous humour samples

Multiplex PCR analyses confirmed the presence of CMV genomic DNA, but none of the other human herpes viruses (HSV-1, HSV-2, VZV, EBV, HHV-6, HHV-7 or HHV-8) in all 11 of the patients (table 2).

Quantitative real-time PCR detected significant viral loads of CMV genomic DNA in the aqueous humour of all 11 patients, with values ranging from 5.4×10^3 to 5.9×10^6 copies/ml (table 2). The mean values for the CMV viral load in the iridocyclitis and corneal endotheliitis groups were 9.4×10^5 and 1.2×10^6 copies/ml, respectively. The differences in CMV viral load between the two groups were not significant (p=0.571).

The corneal endothelial cell damage intensity was correlated to the CMV viral load in the aqueous humour. Results of the linear regression analysis demonstrated a positive correlation between the CMV viral load and the corneal endothelial cell loss (Spearman's correlation coefficient by rank test, r=0.664; p=0.036; figure 3).

However, there was no correlation between the interval from the disease onset to the aqueous sampling and the viral load in the aqueous humour (Spearman's correlation coefficient by rank test, r=0.445; p=0.159). Furthermore, the interval from the disease onset to the sampling was not correlated with the corneal endothelial cell damage intensity (Spearman's correlation coefficient by rank test, r=0.373; p=0.239). In addition, there was also no correlation between the viral load and many other ocular findings, such as cells and flare in the anterior chamber, types of KPs, gonioscopic findings, IOP and post-treatment BCVA.

DISCUSSION

The present study analysed ocular manifestations and CMV viral loads in the aqueous humour of patients with CMV-associated iridocyclitis and corneal endotheliitis. Our major findings included: (1) presence of significant corneal endothelial cell loss in both corneal endotheliitis and iridocyclitis tested eyes; and (2) a significant correlation between corneal endothelial cell loss and CMV viral load in the aqueous humour.

Even though it has been demonstrated that viral infections play a significant role in many inflammatory diseases, a qualitative PCR method that is capable of determining the pathological role of these viral infections has yet to be elucidated. If the presence of viral DNA in an affected disease site could be proven, the quantitative determination and correlation with the clinical manifestations of the viral infection could lead to a much deeper understanding of the role of the virus as a pathogenic disease candidate. For example, we have previously reported on two intraocular inflammatory disorders: one involving uveitis associated with human T-cell leukaemia virus type 1 (HTLV-1)[13 14] and the other involving anterior uveitis associated with VZV.[14] In HTLV-1 uveitis, a significantly higher HTLV-1 viral load was detected in the peripheral blood mononuclear cells of the patients compared with asymptomatic HTLV-1 carriers.[13] This viral load was significantly correlated with the vitreous inflammation of the disease.[14] In our report on anterior uveitis associated with VZV, we demonstrated there was a high VZV viral load within the patient's aqueous humour. Furthermore, there was a significant correlation between the viral load and the intensity of the iris atrophy in these patients.[15]

Table 2 Virological analysis and corneal endothelial cell findings in patients with CMV anterior uveitis

Case	Herpes virus DNA		Endothelial cell count (cells/mm^2)		Corneal endothelial cell loss (%)†	Post-treatment BCVA	Interval from onset to sampling (months)
	CMV (copies/ml)	Others*	Affected eye	Fellow eye			
1	2.3×10^5	-	642	2738	77	0.4	96
2	5.5×10^3	-	1633	2869	43	0.8	8
3	1.3×10^4	-	1695	2789	39	1.5	48
4	6.5×10^4	-	1618	3576	55	1.5	24
5	3.5×10^5	-	1445	2608	38	1.2	14
6	5.9×10^6	-	919	2288	45	1.2	16
7	5.4×10^3	-	2512	3917	60	1.2	6
8	1.0×10^6	-	573	2427	76	0.6	12
9	2.8×10^4	-	1427	2262	35	0.7	5
10	1.2×10^4	-	593	2092	72	0.3	4
11	3.6×10^6	-	620	2674	77	1.5	20

Using aqueous humour samples, genomic DNA of the human herpes viruses was measured by qualitative multiplex PCR and quantitative real-time PCR. Corneal endothelial cell count was examined by specular microscopy.
*Herpes viruses excluding CMV, ie herpes simplex virus type 1 and type 2, varicella zoster virus, Epstein–Barr virus, and human herpes virus types 6, 7 and 8.
†Corneal endothelial cell loss was calculated as described in the methods section.
BCVA, best-corrected visual acuity (decimal fraction); CMV, cytomegalovirus.

Although we found that there was a positive correlation between the corneal endothelial cell loss and the CMV viral load in the aqueous humour, there was no correlation between the viral load and many other ocular signs such as cells and flare in the anterior chamber, types of KPs, gonioscopic findings, IOP, post-treatment visual acuity and the interval from the disease onset to the aqueous sampling. These patients had been treated with topical corticosteroids (eg betamethasone) and anti-glaucoma agents (eg timolol and latanoprost) before they were referred to us by local ophthalmologists. These treatments are known to reduce the intensity of anterior uveitis, IOP and other ocular manifestations, but have no effect on recovering the corneal endothelial cell damage, because the corneal endothelial cell damage is barely reversible.

The cells and flare in the anterior chamber were mild in all 11 patients. A possible explanation why the intensity of the inflammatory reaction in the anterior chamber was so mild in this disease might be related to the involvement of the anterior chamber-associated immune deviation (ACAID).[16 17] In an experimental rabbit corneal endotheliitis model, eyes inoculated with inactivated HSV-1 prior to an active HSV-1 infection exhibited less severe inflammatory reactions and corneal endotheliitis. In addition, they also developed an immune deviation to HSV-1.[18] Although CMV-related ACAID has not been previously reported, real-time PCR in the present study demonstrated that CMV genomic DNA was present at high levels within the anterior chamber of the patients. Therefore, it may be that ACAID in response to CMV occurs in the eye, resulting in a relatively mild inflammatory reaction.

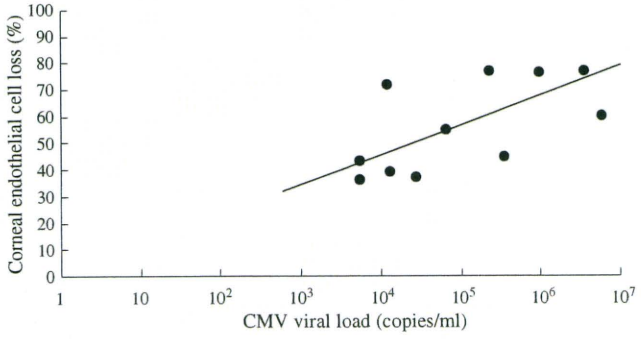

Figure 3 Correlation between cytomegalovirus (CMV) viral load and corneal endothelial cell damage. The CMV viral load was plotted on a logarithmic graph versus the corneal endothelial cell loss (%). The scatter plot shows significant correlation between the CMV viral load and the corneal endothelial cell loss (Spearman's correlation coefficient by rank test, r=0.664; p=0.036).

While our results showed CMV infection in the anterior segment of the eye caused inflammation and corneal endothelial cells loss in immunocompetent hosts, our study cannot answer many other questions. For example, why does CMV cause intraocular inflammation in immunocompetent hosts? Where does the CMV that is detected in the aqueous humour come from? And how is CMV able to cause inflammatory disorder only within the anterior segment of the eye? One possible explanation why our patients developed CMV anterior uveitis is that all our patients had been given topical corticosteroids for a long period of time. This may have contributed to induce local immunosuppressive condition in the anterior segment of the eye and resulted in reactivation of CMV.[8] Further clinical and experimental investigations are necessary to clarify these important questions.

In conclusion, significant corneal endothelial cell damage was detected in all CMV-associated iridocyclitis- and corneal endotheliitis-tested eyes. In addition, a significant correlation was found between corneal endothelial cell loss and the CMV viral load in the aqueous humour.

Competing interests None.

Ethics approval This study was conducted with the approval of the Institutional Ethics Committee of Tokyo Medical and Dental University.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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眼病変にmethotrexate硝子体内注入が著効したが早期に中枢神経へ進展した原発性眼内リンパ腫

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症例報告

眼病変にmethotrexate硝子体内注入が著効したが早期に中枢神経へ進展した原発性眼内リンパ腫

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症例は57歳男性。霧視出現後約5か月で当院を紹介された。右硝子体混濁と網膜の増殖性病変を認め，硝子体液細胞診はClass IIであったがIgH再構成とIL-10/IL-6比の高値を認め全身検索で他部位に病変は認めず原発性眼内リンパ腫（PIOL）と診断した。硝子体へのMethotrexate（MTX）局所注入療法（局注）が著効した。治療開始2か月後，左眼に病変が出現，硝子体液細胞診でClass Vの大型異型リンパ球を認めた。左眼病変もMTX局注で軽快した。しかし2か月後，眼病変出現から10か月後，左前頭葉腫瘍が出現，生検でdiffuse large B-cell lymphomaと診断，MTX大量療法を施行したが進行し全経過約1年8か月で死亡した。PIOLの病理診断は困難であるが本例は比較的早期に診断し得，MTX局注が眼病変に著効した。しかし局注のみでは不十分と考えられ今後適切な治療法の開発が必須である。（臨床血液 **50**（3）：182～186，2009）

Key words : Primary intraocular lymphoma, Intraocular methotrexate injection, IL-10/IL-6 ratio, IgH rearrangement

緒 言

原発性眼内リンパ腫（primary intraocular lymphoma，以下PIOL）は硝子体，網脈絡膜に発症する悪性リンパ腫である。病理診断が困難で，60～80％の患者が中枢神経へ病変が進展[1]，生存中央値は12から20か月と予後不良である[2]。我々は硝子体液中の細胞診に先行してIL-10/IL-6濃度比の上昇，polymerase chain reaction（PCR）法による免疫グロブリン重鎖（IgH）遺伝子の再構成から，症状出現後6か月でPIOLと診断しえた症例を経験した。硝子体へのMethotrexate（MTX）局所注入療法（局注）が著効したが，治療開始後4か月，眼症状出現後10か月で中枢神経に病変が進展した。以上の経過を報告し，本疾患の診断治療上の問題点について考察する。

症 例

症 例：57歳，男性。

主 訴：霧視。

既往歴，家族歴：特記すべき事なし。

現病歴：右眼の霧視出現後4か月で近医を受診しぶどう膜炎と診断された。Betamethasone，levofloxacin点眼を開始したが眼底所見が増悪した。1か月後当院眼科を紹介され精査のため受診した。受診時の右眼底所見を図1に示す。硝子体混濁と網膜の滲出斑および増殖性病変を認め，サルコイドーシスを考えpredonisolone 40 mg/日の経口投与を1か月間行ったが無効であった。眼底の滲出斑が増悪したため，ステロイド抵抗性のぶどう膜炎として悪性リンパ腫を疑い，生検目的の硝子体切除術をおこなう為に当院眼科に入院した。

眼科入院時所見：右眼硝子体切除によって硝子体液を採取し検査を施行したところ細胞診はClass IIであったがIL-10は24,080 pg/ml，IL-6は11,298 pg/mlとIL-10優位のサイトカインの著明な上昇を認めIL-10/IL-6比は約2（>1）であった。眼内リンパ腫を強く疑い，硝子体液から抽出したDNAに対しPCR法でIgHのVDJ geneの再構成の有無を検討したところ，図2Aに示すように免疫グロブリン重鎖の遺伝子再構成を認めた。Primerおよび PCR条件はChhanabhaiら[3]の報告に従った。骨髄，髄液検査，脳核磁気共鳴画像（magnetic resonance imaging，以下MRI）では異常所見はなくHIV抗体は陰性であった。

受付：2008年6月3日
受理：2009年1月8日
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図3に眼科入院後経過を示す。以上の結果からPIOLと診断，病変が急速に進行し黄斑部に接近し失明の危険があったためMTX 400 μgの硝子体局注を3回行ったところ眼底所見は軽快しIL-10は感度未満，IL-6は159 pg/mlと著しく低下し視力も改善した。その後左眼にも硝子体混濁が出現し右眼治療後約2か月後左眼硝子体切除を施行，硝子体液細胞診にて大型異型リンパ球を認めClass Vを得た（図2B）。IL-10，IL-6は43,479 pg/ml，1,245 pg/mlで，両者の比は35であった。MTX局注を3回行い視力は軽快，約2か月後に眼病変も改善をみた。しかし全身化学療法を予定し，治療前評価のために行ったMRIにて左前頭葉にenhanced tumorを認めた（図4A）。開頭生検を施行したところ大型の異型リンパ球のびまん性の浸潤を認め，それらはCD20陽性であった（図4B, C）。以上からDiffuse large B cell lymphomaと診断し血液内科に転科，MTX，vincristine（VCR），procarbazine（PCZ）による全身化学療法（MTX 3,500 mg/m²：day 1, 15，VCR 1.4 mg/m²：day 1, 15，PCZ 100 mg/m²：day 1-7）を3コース施行，MRIにて寛解を確認した。患者の希望で退院，経過観察としていたところ，その約2か月後に左前頭葉に再発を認めた。眼内に再発は認めなかった。全脳照射を施行したが腫瘍の消失にはいたらず照射終了後2か月，全経過約1年8か月で死亡した。

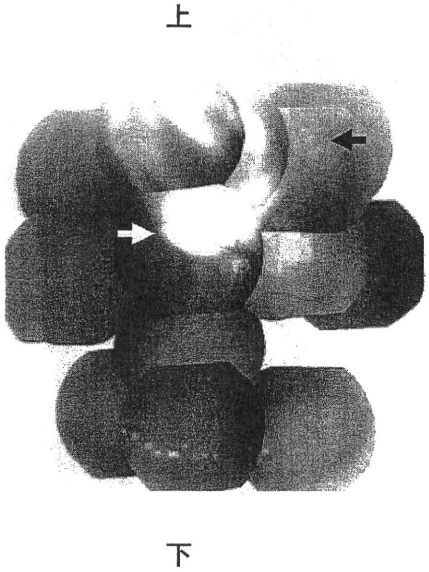

図1 受診時右眼底所見
硝子体混濁による眼底血管の不明瞭化を認めた。網膜に滲出斑（黒矢印），および網膜下増殖性病変（白矢印）を認めた。

考 察

PIOLの発症頻度は非ホジキンリンパ腫の1%と非常にまれである[4]。典型的な症状は無痛性視力低下，視野中の浮遊物で，眼所見は硝子体混濁を伴う後部ぶどう膜炎を呈する[2]。

上記の症状からPIOLを疑った場合，診断は硝子体切除術により採取された硝子体液の細胞診で行われるが，その陽性率は低い。Chanらは硝子体細胞診を施行した54例中，病理診断しえた例は31例（57%）にとどまっていたと報告している[1]。当院眼科でPIOLと診断された5例のうち，細胞診陽性例は本例のみであった[5]。十分な検体量が得られないこと，多くがステロイドの先行投与を受けているため細胞の変性が強いことが原因と思われ

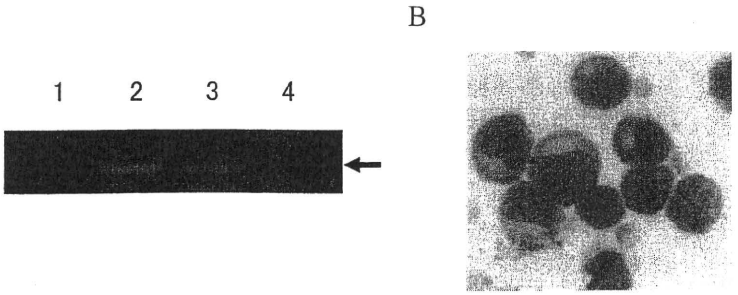

図2 A）右硝子体液PCR検査
単クローン性免疫グロブリン重鎖再構成を認めた。
Lane 1: 陰性コントロール
Lane 2: 陽性コントロール（Raji細胞）
Lane 3: 本症例
Lane 4: 非腫瘍性ぶどう膜炎症例
B）左硝子体液細胞診
大型の異型リンパ球を認めた。（May-Giemsa染色，×600）

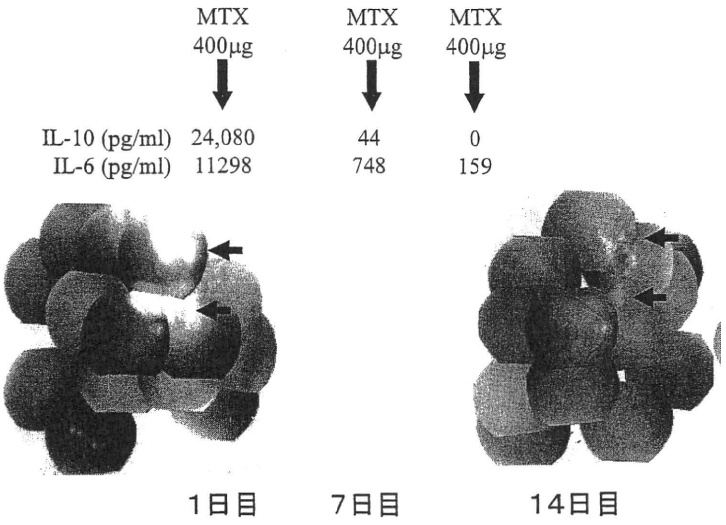

図３　眼科入院後の右眼病変臨床経過
写真は methotrexate 硝子体局所注入前後の眼底所見を示す。注入前後で，硝子体混濁が著明に改善し血管が明瞭に観察されるようになった。増殖性病変（矢印）も著明に改善した。

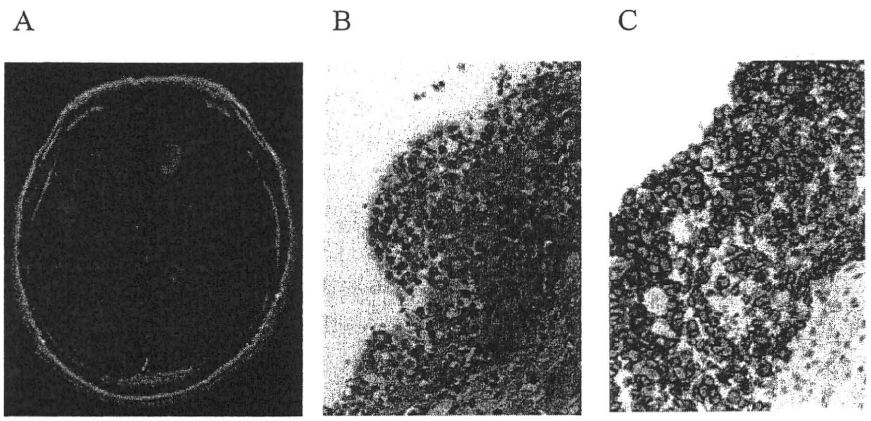

図４　左前頭葉病変
A）Gadolinium 造影 T1 強調核磁気共鳴画像
B）および C）左前頭葉腫瘍病理所見（B: Hematoxylin-Eosin 染色，×400，C: CD20 免疫染色，×400）

る[6]。本例も当院初診時の右眼の結果は Class Ⅱ であった。近年硝子体液中のサイトカイン IL-10/IL-6 比（1.0 以上）[7]や，フローサイトメトリー[8]，PCR 法による免疫グロブリン重鎖（IgH）遺伝子の再構成によるクロナリティーの検索[1]が早期診断のため有用であると報告されている。本症例でもフローサイトメトリーは提出できなかったが，細胞診陰性であった右眼において IL-10/IL-6 比 1.0 以上，IgH 再構成が確認でき，それに基づき治療を開始し，眼所見の改善をみた。以上から PIOL の診断は細胞診を基本に，フローサイトメトリー，PCR 法による IgH の再構成，IL-10/IL-6 の比から総合的に診断すべきと思われる。診断率の向上は予後の改善にもつながると考えられ，そのためには以上の因子を組み込んだ診断基準の設定も必要と思われる。

PIOL はまれな疾患であることから至適治療法は確立されていない。眼局所療法は眼病変への効果が迅速で確実である。従来放射線治療が多く行われていたが，有害事象が多く再発時に再施行できないという問題点があった[6]。近年行われるようになった MTX 局注は重大な有害事象が無く，くり返し投与が可能であることに加え，施

行例全例で有効であったとの報告[9~11]をみる。本例は進行した視力障害に対してMTX局注を行い著明な改善を見た。しかし，MTX局注後の経過としてde SMETらの報告ではほとんどの例が[10]，当院での結果では7例中4例（未発表データ）が，眼病変の寛解にもかかわらずCNSリンパ腫へ進展している。本例もMTX局注により眼病変が改善した2か月後，CNSへ病変の進展をみた。以上からこれら局所療法のみではPIOLの治療は不十分と思われる。

眼は発生学的にCNSの一部と考えられることからPIOLはCNSリンパ腫の一亜型として治療されることも多い。しかし，全身化学療法は眼病変に対し必ずしも有効ではない。Batchelorら[12]は9例の眼内リンパ腫症例（眼内限局1例，CNS病変を伴うもの8例）に対し，大量MTX療法（MTX 8 g/m^{2}を28日ごと，11回投与）を行い，眼病変とCNS病変への効果を検討，CNS病変は保有する7例全例で寛解を得た。しかし眼病変に対しては9例中2例は無効で，効果を認めた7例中3例も眼内に再発した。以上からPIOLに対する治療として局所療法のみならず，全身化学療法も単独では不十分であると考える。本例のように早期にCNSリンパ腫への進展を見る例もあることからも，今後は同時併用も含めた両者を組み合わせた治療の検討が必要と考える。

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