Evidences for Endotoxin as a Causative Factor for Leptospiral Uveitis in Humans

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ABSTRACT

PURPOSE. To understand the pathogenic mechanism of leptospiral uveitis by determining the profile of infiltrating cells, the levels of cytokines and the causative factor in aqueous humor (AH).

METHODS. AH and blood samples were collected from twenty two leptospiral uveitis patients, confirmed by Microscopic Agglutination Test (MAT). Nine Behcet’s, ten phacolytic uveitis and thirteen age-related cataract patients were included as controls. Cytometric Bead Array was used to estimate human inflammatory and Th1/Th2 cytokines. The level of endotoxin in AH was estimated by Limulus Amebocyte Lysate (LAL) test and by dot blot analysis using leptospiral serovar lipopolysaccharide (LPS) specific monoclonal antibody.

RESULTS. Except for one leptospiral uveitis patient, AH from all other patients and controls were negative for Gram negative bacterial endotoxin by LAL test. However, a significant level of serovar Copenhageni LPS was observed in AH of leptospiral uveitis patients seropositive for the same serovar by MAT in contrast to its absence in all controls. A selective infiltration of neutrophils as well as a significant increase in the levels of protein and cytokines - IL-12p70, TNF, IL-6, IL-8 and IL-10 was observed in AH of leptospiral uveitis patients. Phacolytic uveitis was associated with a high proportion of “activated” macrophages and increased levels of IL-6 and IL-8, while Behcet’s uveitis with a predominant infiltration of neutrophils and increased levels of IFN-γ.

CONCLUSIONS. We have demonstrated the presence of serovar specific LPS in AH and thus it is likely that endotoxin is a causative factor for leptospiral uveitis.
INTRODUCTION

Leptospirosis is an acute febrile illness caused by the spirochaete of the genus *Leptospira*. It is a potentially epidemic disease, commonly found in the tropical countries with a humid climate. It can cause both life and vision threatening complications. Uveitis develops as a late complication of the systemic illness in 40% of patients and has been reported even one year after acute illness.¹ A major post monsoon epidemic outbreak of leptospiral uveitis was reported from southern India in 1993. These patients had acute, anterior or pan, non-granulomatous uveitis with hypopyon.² The aetiology of leptospiral uveitis was confirmed by demonstrating the presence of specific anti-leptospiral lipopolysaccharide (LPS) antibodies in the serum of leptospiral uveitis patients.³,⁴ However, the reason for the occurrence of acute ocular inflammation several weeks after recovery from acute systemic illness is not known.

Several animal models are available to understand the pathogenic mechanism associated with the development of uveitis in humans. One such model is the endotoxin induced uveitis (EIU), where systemic or intraocular injection of *Salmonella typhi* LPS migrates into anterior chamber possibly through the iris-ciliary body resulting in a predominant infiltration of neutrophils and macrophages. The severity of uveitis is associated with elevated mRNA expression of TNF-alpha, IL-1 beta, IL-6, IFN-γ, MCP-1 and MCP-2 in iris and ciliary body.⁵,⁶ Further, intraocular injection of TNF, IL-1, IL-2, IL-6 or IFN-γ was shown to induce ocular inflammation in experimental animals.⁷,⁸ However, in
humans, the pathogenic mechanism in uveitis associated with systemic infection and the causative factors are still undefined. The purpose of the present study was to determine the profile of infiltrating cells, the levels of the different cytokines and the lipopolysaccharide (LPS) in AH of leptospiral uveitis patients.

**MATERIALS AND METHODS**

**Recruitment of Cases**

Patients attending the Uvea Clinic, Aravind Eye Hospital, with a clinical diagnosis of leptospiral uveitis were recruited for the study. Demographic information on age, gender, place of residence and socioeconomic status was collected for each patient. After a preliminary examination by a non-ophthalmologist physician and a general ophthalmologist, all patients had a standard uveitis work up. To identify the patients with specific uveitis diagnosis, laboratory and ancillary investigations were tailored for each patient as determined by history and physical findings on presentation. Anatomical location of the inflammation was assigned based on International Uveitis Study Group criteria. Established diagnostic criteria were used to rule out other aetiological diagnosis including HLA B27 related uveitis, Behcet’s syndrome, sarcoidosis, syphilis, tuberculosis, leprosy, acute retinal necrosis, VKH syndrome, sympathetic ophthalmia and others. Cases were classified under idiopathic group when a specific diagnosis was not known and were not included in the present study.

Twenty two patients diagnosed as leptospiral uveitis based on a specific combination of clinical features and a positive serology by MAT were included in
The study.\textsuperscript{4} Ten phacolytic uveitis with hypopyon, nine acute Behcet’s uveitis, three Fuch’s heterochromic cyclitis, two sarcoidosis patients were selected as non-leptospiral uveitis controls. Thirteen uncomplicated, age-related cataract patients who showed no symptoms of systemic or ocular infection were also included as controls. This study was approved by the Institutional Review Board of Aravind Eye Hospital, which adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all the patients before recruitment. About 100 µl AH was collected from leptospiral uveitis patients by anterior chamber paracentesis in the operation theatre. Aqueous samples from phacolytic uveitis, Behcet’s uveitis and cataract patients were collected at the time of surgery. Blood (5 ml) was obtained from all cases for serological analysis and smear preparation. All sera were stored at –80°C.

**Materials**

Twenty leptospiral serovars and the monoclonal antibody F70 24-15 were obtained from Royal Tropical Institute, The Netherlands. Cytometric Bead Array (CBA) kits for human inflammatory cytokines (IL-8, IL-1β, IL-6, IL-10, TNF and IL-12) and Th1/Th2 cytokines (IL-2, IL-4, IL-5, IL-10, TNF-α and IFN-γ) were purchased from Beckton Dickinson Biosciences Pharmingen, San Diego, CA; biotinylated goat anti-mouse Ig was obtained from Dako cytomation (Denmark, A/S); streptavidin-HRP, 4-CN, BSA, Bradford reagent, Folin Ciocalteau reagent from Sigma Aldrich (St. Louis, MO) and nitrocellulose (NC) from Amersham Pharmacia Biotech (Germany). QCL-1000 Chromogenic Limulus Amebocyte
Lysate (LAL) was purchased from Cambrex Bio Science Walkersville, Inc., MD, USA and LAL reagent water from Salesworth India Pvt. Ltd., Bangalore, India.

**Microscopic Agglutination Test (MAT)**

All the serum samples were tested for the presence of anti-leptospiral antibodies by MAT. A panel of 20 serovars of *Leptospira sp.* was used as antigen and the end titre was defined as the highest dilution of serum having 50% agglutination. MAT was considered positive at 1:100 dilution of serum.\(^4,12\)

**Cytospin**

Cells in AH were separated by centrifugation at 2000 rpm for 10 minutes at 4°C. The supernatant was transferred to a fresh tube and stored at –80°C for cytokine analysis. The cell pellet was resuspended in 200 \(\mu\)l of phosphate buffered saline (PBS) and deposited onto glass slides in a Shandon Cytospin 3 by centrifuging at 400 rpm for 3 minutes. After air drying, the blood smear and cytospin preparations were stained with Giemsa. The nature of infiltrating cells was then analyzed and a minimum of 200 cells were counted for each sample.

**Cytokine analysis**

The levels of human inflammatory cytokines (IL-8, IL-1\(\beta\), IL-6, IL-10, TNF and IL-12) and Th1/Th2 cytokines (IL-2, IL-4, IL-5, IL-10, TNF-\(\alpha\) and IFN-\(\gamma\)) in AH and serum were determined by flow cytometry using CBA as per the manufacturer’s instructions. Briefly, the six capture bead populations with distinct fluorescence intensities that were coated with cytokine specific capture antibodies were mixed together in equal volumes. To 50 \(\mu\)l of the mixed bead population, 50 \(\mu\)l of the recombinant standards (20 to 5000 pg/ml) or AH or serum samples and 50 \(\mu\)l of
PE-conjugated detection antibodies were added and incubated together for three hours at 25°C in dark to form sandwich complexes. The beads were then washed with the wash buffer and the sample data were acquired using flow cytometer (BD FACS Calibur) with the BD CBA Software. The standard curve and sample results were generated in graphical and tabular format.

**Protein estimation**

The protein concentration was determined in AH and serum by the method of Bradford and Lowry respectively using bovine serum albumin as standard.

**Chromogenic LAL Test**

The level of Gram negative bacterial endotoxin was quantified using LAL test as per the manufacturer's instructions. Briefly, 50 µl standard or AH from patients was mixed with LAL supplied in the kit and incubated at 37°C for 10 minutes. The substrate solution was then added and incubated at 37°C for an additional 6 minutes. The reaction was stopped with 25% v/v glacial acetic acid in LAL reagent water. The absorbance was then read at 405 nm using the ELISA reader. A standard curve was constructed using the standard in the range 0.1-1.0 EU/ml and the concentration of endotoxin in each sample was determined. Since we found inhibitory factors in AH, when spiked with the standard, the observed inhibition was overcome by heating the AH at 70°C for 10 minutes, at a dilution of 1:20.

**Leptospiral LPS estimation**

*Leptospira interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni specific monoclonal antibody (F70 24-15) was used to test for the presence of
leptospiral LPS in AH. Antigen containing LPS from five leptospiral serogroups (Australis, Autumnalis, Icterohaemorrhagiae, Louisiana and Patoc) were prepared following the published protocol and used to test for the specificity of the above monoclonal antibody. The leptospiral antigen and AH was transferred to the NC membrane by vacuum filtration method using BIO RAD dot blot apparatus. After blocking with 5% skimmed milk powder in PBS for 2 hours at room temperature, the blots were incubated with monoclonal antibody F70 C24 (1:1000), followed by biotinylated anti-mouse Ig (1:3000), streptavidin HRP (1:1000) in 1% BSA in PBS-Tween 20 (PBS-T) for 1 hour each. Blots were washed with PBS-T after each incubation and developed with 4-CN. The results were read by measuring the intensity/mm² of the dot in Gel-Documentation system (BIORAD). Using different concentrations of the antigen containing LPS from *Leptospira interrogans* serogroup Icterohaemorrhagiae (15 to 125 ng/dot), a standard curve was constructed with intensity/mm² in the X-axis and concentration in the Y-axis. A linear curve was observed between the range 31 ng to 125 ng/dot, from which the unknown concentrations were calculated using BIORAD Quantity One software. Since the volume of AH collected from uveitis patients varied, it was not possible to load 100 µl in each uveitis sample. However, the concentration of LPS in all the samples was determined using the standard curve and extrapolated for 100 µl.

Statistical Analysis
Analysis and graphical representation of the data was carried out using Stata 8.2 (Stata, College Station, TX) software and Microsoft Excel. The data were tested using Mann Whitney Rank Sum test with a significance level of 0.05.

RESULTS

All the twenty-two leptospiral uveitis patients were serologically confirmed by MAT for leptospiral infection. They were positive for pathogenic \textit{L. interrogans} serovars Copenhageni (8), Icterohaemorrhagiae (2), Autumnalis (3), Australis (2), Andamana (1), Hardjo (1), Louisiana (1) and to the saprophytic \textit{Leptospira biflexa} serovar Patoc (4). All the non-leptospiral uveitis and cataract patients recruited for the study were negative for leptospiral antibodies.

Protein exudation in AH

A significant (p<0.01) increase in the level of protein was observed in AH of leptospiral (median [min, max]: 5.3 mg/ml [0.95, 53]), phacolytic (10 mg/ml [3, 46.4]) and Behcet’s uveitis patients (13.94 mg/ml [2.0, 33.4]) compared to cataract patients (0.5 mg/ml [0.1, 0.8]). However, the serum protein levels were same in all the four groups (Fig. 1).

Selective infiltration of neutrophils

A significantly higher proportion (p<0.001) of neutrophils was observed in AH of leptospiral and Behcet’s than in phacolytic uveitis patients (Table 1). The latter group was distinctly different from others on the basis of macrophages as predominant infiltrating cells. Comparison of cellular profile in AH and corresponding blood samples indicated a selective infiltration of neutrophils in AH
of leptospiral and Behcet’s and macrophages in phacolytic uveitis patients. No cells were observed in AH of all the cataract controls (Table 1).

3 **Cytokines in AH and Serum**

Fifty microlitre of undiluted AH or serum samples were analysed for human inflammatory and Th1/Th2 cytokines by CBA. A significantly higher concentration of IL-6, IL-8, IL-12p70, TNF and IL-10 was observed in AH of leptospiral uveitis patients than in cataract controls (Fig. 2). Further, leptospiral uveitis was also significantly different from other uveitis in IL-12p70, TNF and IL-10. IL-6 levels were similar in all uveitis.

As shown in Figure 3, we did not observe increased levels of Th1/Th2 cytokines in leptospiral uveitis patients. Interestingly, the signatory Th1 cytokine IFN-γ, was significantly higher in Behcet’s uveitis than in cataract controls. The levels of the inflammatory/Th1/Th2 cytokines in serum were below the detection limit in both leptospiral uveitis patients and controls.

15 **Level of Endotoxin in AH**

16 **LAL estimation:** AH samples from five leptospiral uveitis patients seropositive by MAT, three Behcet’s uveitis and three cataract patients were tested for the presence of endotoxin by LAL assay. Except for one AH (0.656 EU/ml) from a leptospiral uveitis patient, all were negative for Gram negative bacterial endotoxin.

18 **Dot Blot analysis:** Antigenic preparations containing LPS from different serogroups were used to test for the specificity of the monoclonal antibody for *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni. A positive
reaction was observed only with antigen from serovar Copenhageni (Ictero-Cop) and not with other serogroups indicating the specificity of the antibody (Fig. 4A). Further, the positive reaction remained after treating the antigen with proteinase K (Fig. 4B); but was totally lost after periodate treatment (Fig. 4C) confirming that LPS is the antigen detected by this monoclonal antibody. High levels of serovar specific LPS was observed in AH of leptospiral uveitis patients (Figs. 5, 6) and interestingly, the sera of these patients were MAT positive for the same serovar Copenhageni.

DISCUSSION

Though there are animal models to understand the pathogenic mechanism associated with the development of uveitis, only little information is available on humans. Most of these reports are based on analysis of the cytokine levels in a heterogenous group of uveitis patients, only a few have analyzed the levels in defined entity of uveitis. The present study is the first of its kind in selecting a homogenous group of patients with confirmed leptospiral aetiology to identify the profile of cytokines in AH and to identify the associated causative factor. The aetiology in all the recruited leptospiral uveitis cases was confirmed by MAT, the gold standard test for leptospirosis even in a geographic region like India where it is more common. We have demonstrated that MAT positivity is due to IgM antibodies towards leptospiral LPS. Analysis of AH and corresponding blood samples from leptospiral uveitis patients revealed: (1) protein exudation, the first sign in the break-down of blood-
aqueous barrier\textsuperscript{23}, (2) a selective infiltration of neutrophils into AH in contrast to that observed in autoimmune uveitis, mediated by T-cells\textsuperscript{20} and (3) a higher level of inflammatory cytokines – IL-12p70, IFN-\(\gamma\), TNF, IL-6, IL-8 and the regulatory cytokine IL-10 in AH compared to their serum samples, indicating local production. Thus the specific profile of cytokines in AH of leptospiral uveitis patients indicates that it is mediated by inflammatory cytokines, since the signatory cytokines of Th1/Th2 were not observed. Further, \textit{in vitro} studies have shown that heat-killed leptospires were able to induce production of IL-12p40, TNF-\(\alpha\) and IFN-\(\gamma\) in whole blood from healthy volunteers.\textsuperscript{24}

A crucial question on the pathogenesis of leptospiral uveitis is about the nature of causative factor for inducing acute anterior uveitis, especially when there were no clinical symptoms of systemic leptospirosis at the time of presentation at the Uvea Clinic. The results on the cellular and cytokine analysis in leptospiral uveitis patients correlate well with the findings in EIU\textsuperscript{25} but not with experimental autoimmune uveoretinitis (EAU) animal model.\textsuperscript{26} The positive correlation is based upon the protein exudation, neutrophil infiltration as well as the local production of cytokines – IL-12p70, IFN-\(\gamma\), TNF, IL-6, IL-8 and IL-10 in AH, synthesized by iris and ciliary body.\textsuperscript{5,27} Therefore, it is possible on the basis of the above findings that leptospiral LPS might be the initiating factor for the development of acute anterior uveitis, several weeks to months after systemic infection. There are reports to suggest that LPS from circulation migrates into the anterior chamber possibly through iris-ciliary body at a concentration (1- 10 ng) sufficient to induce inflammatory response.\textsuperscript{28,29}
Leptospiral LPS is 10-fold less toxic compared to *S. typhi* LPS used in EIU models and other Gram negative bacterial LPSs, and its reduced toxicity is due to the absence of β-hydroxy myrsitic acid. But it has been demonstrated to activate macrophages *in vitro*. This might be the probable reason for the low sensitivity of LAL assay, a functional assay of LPS toxicity, in leptospiral uveitis patients. To overcome this, a method for estimating the level of leptospiral serovar specific LPS was developed in this study using a monoclonal antibody specific for *L. interrogans* serovar Copenhageni LPS O-antigen. Interestingly, higher levels of serovar specific LPS was observed in leptospiral uveitis patients and these patients were also seropositive for the same serovar Copenhageni. These results indicate that the leptospiral LPS in the AH is from the original infecting organism. Further, Behcet’s uveitis and cataract controls were negative for Gram negative endotoxin in their AH. Therefore, demonstration of a significant concentration of infecting serovar specific LPS in AH suggests that leptospiral uveitis is endotoxin mediated. However, the source and pathological effect of leptospiral LPS in the AH need to be elucidated.

Behcet’s disease is a chronic inflammatory disorder characterized by recurrent attacks of different clinical manifestations, including oral ulcers, genital ulcers, uveitis, skin lesions, arthritis, venous thrombosis, arterial aneurysms, and lesions in the central nervous and gastrointestinal systems. The aetiology of Behcet’s disease is unknown, but is considered to be chronic, autoimmune uveitis. In the present study, a predominant infiltration of neutrophils and a higher
concentration of IFN-γ were observed in AH of Behcet’s uveitis, confirming the earlier reports of Shimada et al., (1971) \(^ {34}\) and Lacomba et al. (2000). \(^ {17}\)

Phacolytic uveitis, caused by the leakage of the lens proteins was included in the present study as uveitis control of non-infectious type. Accordingly, leptospiral uveitis could be distinguished from phacolytic uveitis on the basis of the pattern of infiltrating cells\(^ {35}\) and cytokines.

Evidences are made available in this study to distinguish leptospiral uveitis from phacolytic uveitis and Behcet’s uveitis (Table 2). The specific combination of clinical feature in leptospiral uveitis\(^ {9}\) along with laboratory confirmation of leptospiral aetiology, the nature of infiltrating cells and profile of cytokines collectively indicate that leptospiral uveitis is a distinct entity, different from phacolytic, Behcet’s and possibly other forms of uveitis.

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1 References


Table 1. Profile of infiltrating cells in AH and blood of leptospiral uveitis patients and controls

<table>
<thead>
<tr>
<th>Cellular Profile</th>
<th>Neutrophils (%)</th>
<th>Lymphocytes (%)</th>
<th>Monocytes (%)</th>
<th>Macrophages (%)</th>
<th>Eosinophils (%)</th>
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<tr>
<td><strong>AQUEOUS HUMOR</strong></td>
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<tr>
<td>Leptospiral Uveitis (10)</td>
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<td>12 (1,34)</td>
<td>2 (0,7)</td>
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<td>Phacolytic Uveitis (10)</td>
<td>2 (0,18)</td>
<td>8 (0,13)</td>
<td>2 (0,5)</td>
<td>87 (70,90)</td>
<td>0</td>
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<tr>
<td>Behcet’s Uveitis (6)</td>
<td>85 (48,91)</td>
<td>9 (1,15)</td>
<td>2 (0,2)</td>
<td>0 (0,3)</td>
<td>0</td>
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<tr>
<td>Cataract Controls (10)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td><strong>BLOOD</strong></td>
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<tr>
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<td>34 (21,62)</td>
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<td>2 (0,4)</td>
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<td>2 (0,7)</td>
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<tr>
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<td>23 (5,23)</td>
<td>3 (2,5)</td>
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<td>2 (1,6)</td>
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<td>69 (59,76)</td>
<td>25 (22,34)</td>
<td>4 (1,5)</td>
<td>0</td>
<td>2 (1,2)</td>
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</table>

After Giemsa staining, a minimum of 200 cells were counted for each patient.
* Significantly different from AH of phacolytic uveitis patients (p<0.001);
‡ Significantly different from the corresponding blood sample (p<0.005)
<table>
<thead>
<tr>
<th></th>
<th>LEPTOSPIRAL UVEITIS (22)</th>
<th>PHACOLYTIC UVEITIS (10)</th>
<th>BEHCET’S UVEITIS (9)</th>
<th>CATARACT CONTROLS (13)</th>
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<td>Acute</td>
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<td>Chronic</td>
<td>-</td>
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<td>Unilateral</td>
<td>Unilateral / Bilateral</td>
<td>-</td>
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<tr>
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<td>Anterior/ Pan Uveitis</td>
<td>Anterior Uveitis</td>
<td>Pan Uveitis</td>
<td>-</td>
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<tr>
<td>IgM antibodies to leptospiral LPS in serum by MAT</td>
<td>Infectious</td>
<td>Non-infectious</td>
<td>Autoimmune</td>
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<tr>
<td>Protein exudation in AH</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
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<td>Cellular infiltration in AH</td>
<td>Neutrophils</td>
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<td>+++</td>
<td>-</td>
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<tr>
<td></td>
<td>Macrophages</td>
<td>-</td>
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<td></td>
<td>Lymphocytes</td>
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<td></td>
<td>Monocytes</td>
<td>+</td>
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<tr>
<td>Cytokines in AH</td>
<td>IL-6</td>
<td>+++</td>
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<td></td>
<td>IL-8</td>
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<td>IL-10</td>
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<tr>
<td></td>
<td>IFN-γ</td>
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<td>+++</td>
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<tr>
<td>Leptospiral LPS in AH</td>
<td>++</td>
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</table>

+++ - very high level/maximum number
++ - high level
+ - low level/minimum number
± - very low level
- - negative
FIGURE 1. Levels of protein in AH and serum of leptospiral uveitis patients and controls. The lines within boxes indicate the median concentration of protein; hinges on top/bottom of box the upper/lower quartile and horizontal lines above and below the boxes the most extreme values in the sample. *Significantly higher than cataract control (p<0.001). LU-Leptospiral Uveitis; PU-Phacolytic Uveitis; BU-Behçet’s Uveitis; CC-Cataract Controls.
FIGURE 2. Inflammatory cytokines in AH of leptospiral uveitis and controls. Fifty microlitre undiluted AH was analysed by CBA for human inflammatory cytokines. * Significantly higher compared to cataract control (p< 0.05); † Significantly higher compared to phacolytic and Behcet’s uveitis (p<0.05). The error bars indicate the minimum and maximum value.
FIGURE 3. Th1/Th2 cytokines in AH of uveitis patients and controls. Fifty microlitre undiluted aqueous humor was analysed by CBA for Th1/Th2 cytokines. * Significantly higher compared to cataract control (p< 0.05); † Significantly higher compared to phacolytic, Behcet's uveitis and cataract control (p<0.05). The error bars indicate the minimum and maximum value.
FIGURE 4. Confirmation that monoclonal antibody F70 24 antibody is specific for *L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni LPS. Leptospiral LPS antigens (1.0 µg/dot) were spotted on to nitrocellulose membrane. Dot blot analysis was performed without treatment (A), after proteinase K treatment (B) and after periodate treatment (C) of antigens using serovar Copenhageni specific monoclonal antibody (1:1000) followed by biotinylated goat anti-mouse Ig (1:3000) and streptavidin-HRP (1:1000). Ictero-Cop: Serogroup Icterohaemorrhagiae serovar Copenhageni.
FIGURE 5. Estimation of Leptospiral LPS in AH by dot blot analysis. (A) Different concentrations of serovar Copenhageni LPS antigen were spotted to prepare the standard curve. (B) Indicated amount of AH of leptospiral uveitis patients seropositive to serovar Copenhageni (LU-1 to LU-5) and (C) AH (100 µl) of cataract controls (CC-1 to CC-5) were used as antigen, followed by Icterohaemorrhagiae LPS specific monoclonal antibody (1:1000), biotinylated goat anti-mouse Ig (1:3000) and streptavidin-HRP (1:1000). Cop – Serovar Copenhageni.
FIGURE 6. Amount of leptospiral LPS in AH of leptospiral uveitis patients and controls (7 samples/group). The level of leptospiral LPS in five AH samples included in Figure 5 along with additional two samples from leptospiral uveitis were quantified and included in this graph. All seven were from patients who were MAT seropositive for L. * Significantly higher than non-leptospiral uveitis and cataract controls (p<0.05).