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The CD4/CD8 Ratio in Vitreous Fluid Is of High Diagnostic Value in Sarcoidosis

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Purpose: Sarcoidosis is an idiopathic inflammatory disorder involving multiple organs, and ocular manifestation (represented by granulomatous uveitis) is one of the common features. A well-known immunologic feature in sarcoidosis is an increased CD4+ helper T-cell type 1 lymphocyte subset in bronchoalveolar lavage (BAL) fluid. The current study investigated the vitreous lymphocyte subsets of ocular sarcoidosis to elucidate the immunologic features of this disorder in the eye.

Design: Case-control study.

Participants and Controls: Fifty-one eyes of 38 patients with ocular sarcoidosis, confirmed by international diagnostic criteria, were enrolled in this study. Twenty-seven eyes of 26 patients with other causes of uveitis were enrolled as nonsarcoid controls.

Methods: Evaluation of diagnostic tests for cell profiles of ocular sarcoidosis. Lymphocytes in the vitreous samples were analyzed by cytology, polymerase chain reaction, and flow cytometry. Peripheral blood was also obtained from each patient and analyzed in comparison with the vitreous samples.

Main Outcome Measures: CD4/CD8 ratios of vitreal and peripheral T lymphocytes.

Results: CD4/CD8 ratios of the vitreous T lymphocytes were significantly higher in ocular sarcoidosis than in nonsarcoidosis vitreous samples. In the patients with ocular sarcoidosis, the CD4/CD8 ratios of vitreal T lymphocytes were significantly higher than the CD4/CD8 ratios of peripheral T lymphocytes. No significant differences were found between the CD4/CD8 ratios of vitreal and peripheral T lymphocytes in the patients without sarcoidosis. Moreover, the CD4/CD8 ratios of peripheral T lymphocytes in the patients with ocular sarcoidosis were significantly higher than in patients without sarcoidosis. The sensitivity and specificity of the vitreal CD4/CD8 ratio were 100% and 96.3%, respectively, for the diagnosis of ocular sarcoidosis.

Conclusions: Our findings suggest that the CD4/CD8 ratio of vitreous-infiltrating lymphocytes has high diagnostic value in ocular sarcoidosis, comparable to that of the CD4/CD8 ratio in BAL fluid lymphocytosis for pulmonary sarcoidosis. Furthermore, a high CD4/CD8 ratio of peripheral blood T lymphocytes should be one of the laboratory findings for ocular sarcoidosis. Diagnostic vitrectomy using flow cytometric analysis may be a useful adjunct for the diagnosis of ocular sarcoidosis, particularly in complex cases.

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Sarcoidosis is an idiopathic inflammatory disorder characterized by noncaseating epithelioid cell granulomas in multiple tissues and organs.[1,2] The disease most commonly affects the lung, but other tissues (e.g., the heart, central nervous system, and spleen) may also be affected.[3] Although the exact cause of sarcoidosis is currently unknown, studies have linked the disease with genetic, environmental, or age-related heterogeneity.[4,5] Both endogenous and pathogenic bacterial species have been detected in sarcoid lesions, although their association with disease etiology remains unclear.[6] It has been surmised that genetic predispositions, compounded by other factors, may trigger granulomatous responses to endogenous or infectious microbes in sarcoidosis.[5,7]

The basic diagnostic criteria for sarcoidosis include the clinical and radiologic presentation, histopathologic verification of noncaseating epithelioid cell granulomas, and exclusion of other diseases with granuloma formation reactions (e.g., tuberculosis).[3] Clinical features suggestive of sarcoidosis include uveitis in the eye, erythema nodosum, and maculopapular skin lesions; chest radiography may reveal bilateral hilar lymphadenopathy (BHL) or pulmonary infiltration. Previous reports have shown that fiberoptic bronchoscopy can be used to enable a transbronchial lung biopsy for the diagnosis of sarcoidosis.[8,9]

Immunologic findings are typical in sarcoidosis.[3] An increased CD4+ helper T-cell type 1 lymphocyte subset (lymphocytosis) in bronchoalveolar lavage (BAL) fluid and a CD4/CD8 ratio greater than 3.5 are helpful for the diagnosis of sarcoidosis. Depression of delayed-type hypersensitivity is also known to be a common feature of sarcoidosis.[10] Bronchoalveolar lavage lymphocytosis (CD4:CD8 >3.5) and other lab-

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oratory features highly consistent with sarcoidosis, such as high serum levels of angiotensin-converting enzyme, can be reliable indicators when diagnosing sarcoidosis in cases without histology.[11,12]

It is reported that 30% to 60% of patients with sarcoidosis have ocular involvement.[13–16] Bilateral anterior or posterior uveitis is common, but the conjunctiva, lacrimal gland, and orbit of the eye also can be affected. The clinical presentation of sarcoid uveitis is characteristically marked by iris nodules, mutton-fat keratic precipitates, and tent-shaped peripheral anterior synechia in the anterior segment of the eye. Phlebitis and vitreitis, resulting in snowball-like vitreous opacity, are the common posterior segment findings. Chronic uveitis can result in the formation of an epiretinal membrane and cystoid macular edema, leading to severe visual impairment.[17] Uveitis is commonly treated with topical (or occasionally systemic) corticosteroids, but in cases accompanied by the formation of an epiretinal membrane or long-standing vitreous opacity, surgical intervention may be necessary.

For the diagnosis of sarcoidosis with ocular involvement, internationally acknowledged criteria have been established.[18] Although it is generally not difficult to diagnose ocular sarcoidosis with typical clinical findings, a considerable number of sarcoidosis cases present with nonspecific vitreal opacity, thus making a differential diagnosis difficult.[19]

Analysis of a vitreous sample is reported to be useful in diagnosing uveitis or other diseases with vitreal opacity. Vitreous samples may be analyzed by cytology, cell surface marker analysis by cytokine measurement, and polymerase chain reaction (PCR) for detection of infectious origins, such as viral, fungal, or bacterial DNA.[20,21] Flow cytometric analysis is also reported to be useful in diagnosing primary intraocular lymphoma[22] and uveitis.[23]

The current study found that the CD4/CD8 ratios of lymphocytes obtained from vitrectomy specimens are significantly higher in sarcoidosis compared with nonsarcoid uveitis. We also discovered that a CD4/CD8 ratio greater than 3.5 of vitreous-infiltrating lymphocytes is highly specific to ocular sarcoidosis and is efficient for the differential diagnosis of the disease. Moreover, a vitreal CD4/CD8 ratio greater than 3.5 was found to have high sensitivity and specificity in the diagnosis of ocular sarcoidosis. This was comparable to a CD4/CD8 ratio greater than 3.5 from BAL fluid for the diagnosis of pulmonary sarcoidosis.

Materials and Methods

The study was designed as a prospective study. Patients who met inclusion criteria were identified in the uveitis outpatient clinic at Kyoto Prefectural University of Medicine and invited to participate in the study. Details of patients and controls are provided in Tables 1 and 2 (available at http://aaojournal.org). Included were patients with uveitis with visual disturbance due to prolonged vitreous opacity or epiretinal membrane that was refractory to conventional steroid therapy; enrolled patients agreed to receive pars plana vitrectomy and to participate in the study. Patients were excluded from the ocular sarcoidosis group if they had a history of intraocular surgery or other active systemic disease. Patients in the study were categorized into 2 groups (sarcoidosis and nonsarcoidosis) according to international criteria for the diagnosis of ocular sarcoidosis established at the International Workshop on Ocular Sarcoidosis (IWOS).[18] The IWOS criteria have 4 classifications of ocular sarcoidosis based on suggestive clinical signs, appropriate laboratory investigations, and biopsy results. Briefly, biopsy-supported diagnosis with a compatible uveitis is classified as "definite" ocular sarcoidosis; presence of BHL and compatible uveitis but without biopsy is classified as "presumed" ocular sarcoidosis; presence of suggestive intraocular signs and 2 positive investigational test results (negative tuberculin skin test result, elevated angiotensin-converting enzyme, elevated liver enzymes, and chest computed tomography) without BHL and biopsy is classified as "probable" ocular sarcoidosis; negative biopsy, with suggestive intraocular signs and 2 positive investigational test results, is classified as "possible" ocular sarcoidosis. Patients who met the IWOS criteria were categorized as the sarcoidosis group, and other patients were categorized as the nonsarcoidosis group.

This study was approved by the institutional review board of Kyoto Prefectural University of Medicine. All experimental procedures were conducted in accordance with the tenets set forth in the Declaration of Helsinki. The study was registered in the University Hospital Medical Information Network Clinical Trial Registry. The purpose of the research and the experimental protocols were explained in detail to all patients, and their informed consent was obtained before participation in this study.

Participants

A total of 78 eyes of 64 patients were enrolled in this study. Of those 78 eyes, 51 eyes of 38 patients were diagnosed with ocular sarcoidosis. Of the 38 patients with ocular sarcoidosis, 15 were diagnosed with "definite" ocular sarcoidosis on the basis of positive histopathologic manifestation by skin biopsy (12 patients) and transbronchial lung biopsy (3 patients). Eleven patients with BHL but without biopsy were categorized as having "presumed" ocular sarcoidosis, and 12 patients without BHL and biopsy were diagnosed with "probable" ocular sarcoidosis on the basis of laboratory investigation. There were no patients diagnosed with "possible" ocular sarcoidosis. The remaining 27 eyes (of 26 patients) were grouped as nonsarcoid controls; in these cases, uveitis was associated with endophthalmitis, acute retinal necrosis, primary intraocular lymphoma, or idiopathic uveitis. The basic characteristics of all patients are listed in Tables 1 and 2 (available at http://aaojournal.org).

Sample Collection Procedures

From each patient, a vitreous specimen was obtained at the start of a conventional 20- or 25-gauge pars plana vitrectomy operation using a CV-24000 (NIDEK, Co., Ltd., Aichi, Japan) or an Accurus (Alcon Laboratories, Inc., Fort Worth, TX) vitrectomy system. A 3-way cock was attached between the connection part of the suction-tube line of the cutter probe, and a 10-ml syringe was connected to the free end of the 3-way cock. Dry vitrectomy without balanced salt solution (Alcon Laboratories, Inc.) perfusion was conducted with a cut rate of 500 cpm so as not to damage cells infiltrating the vitreous. After collecting 1.5 to 3 ml of pure vitreous sample, additional vitrectomy was performed under balanced salt solution perfusion. All of the obtained samples were promptly stored at 4°C and then brought to the clinical laboratory of the university hospital for cell analysis (including flow cytometry). Peripheral blood samples were collected preoperatively from patients, and samples were immediately analyzed with the following experiment.

Procedure of Flow Cytometry Analysis of Vitreous and Peripheral Blood Samples

Vitreous samples were filtered with a 70-μm cell strainer (BD Falcon Cell Strainer; BD Biosciences, Bedford, MA) and then washed and resuspended with phosphate-buffered saline (Nissui, Tokyo, Japan) containing 2% bovine serum albumin (Nacalai Tesque, Kyoto, Japan) and 0.1% sodium azide (Nacalai Tesque) at a final volume of 0.5 ml. For the flow cytometric analysis, 0.1 ml of each sample was incubated with one of the following mixtures of monoclonal antibodies: fluorescein isothiocyanate (FITC)-conjugated mouse immunoglobulin-G1 (Dako Denmark AS, Glostrup, Denmark)/phycoerythrin (PE)-conjugated mouse immunoglobulin-G1 (Dako Denmark AS)/PE-Cy-Chrome5 (Cy5)–conjugated anti-CD45 (clone T/29/33; Dako Denmark AS), anti–CD3-FITC (UCHT1; Dako Denmark AS)/anti–CD19-PE (HD37; Dako Denmark AS)/anti–CD45-Cy5 (T29/33; Dako Denmark AS), or anti–CD4-FITC (T4; Beckman Coulter, Miami, FL)/anti–CD8-PE (T8; Beckman Coulter)/anti–CD3-Cy5 (UCHT1; Dako Denmark AS), respectively. After incubation for 15 minutes at room temperature in complete darkness, the samples were washed again and resuspended at a final volume of 0.5 ml. To examine the lymphocyte subsets of the vitreous samples using an Epics XL-MCL flow cytometer (Beckman Coulter), we initially counted $CD45^+$ cells on CD45 versus side-scatter plots combined with forward-scatter and side-scatter. If more than 100 $CD45^+$ cells were found to be contained in 1 test tube, then further analysis was performed to examine the T-cell ($CD3^+$ $CD45^+$)/B-cell ($CD19^+$ $CD45^+$) ratio, as well as the $CD3^+$ $CD4^+$ T-cell/$CD3^+$ $CD8^+$ T-cell ratio. The remaining portion (0.2 ml) of the resuspended vitreous sample was prepared for cytology with a cytocentrifuge (Cytospin 4; Thermo Fisher Scientific, Inc., Waltham, MA).

Flow cytometry was also used to study the peripheral blood lymphocyte population. Briefly, whole blood anticoagulated with ethylenediaminetetraacetic acid was stained with the same panels of monoclonal antibodies used for vitreous samples, treated with Multi-Q-prep (Beckman Coulter) to lyse the contaminating red blood cells, washed, and resuspended at a final volume of 0.5 ml/tube.

Real-Time Polymerase Chain Reaction Analysis of Vitreous Samples

Genomic DNA of human herpes virus (HHV) in the aqueous humor and vitreous was measured through the use of 2 independent PCR assays (a qualitative multiplex PCR assay and a quantitative real-time PCR assay) as described previously.[24,25] DNA was extracted from samples using an E21 virus minikit (QIAGEN, Inc., Valencia, CA) installed on a robotic workstation for automated purification of the nucleic acids (BioRobot E21; QIAGEN, Inc.). The multiplex PCR was designed to qualitatively measure the genomic DNA of 8 types of HHV and other ocular pathogens: herpes simplex virus (HSV) type 1 (HSV-1 or HHV-1) and type 2 (HSV-2 or HHV-2), varicella zoster virus (HHV-3), Epstein–Barr virus (HHV-4), cytomegalovirus (HHV-5), HHV-6, HHV-7, HHV-8, *Propionibacterium acnes*, *Toxoplasma*, *Toxocara*, *Bartonella henselae*, *Chlamydia trachomatis*, *Treponema pallidum*, *Mycobacterium tuberculosis*, *Candida* (18s rRNA), *Aspergillus* (18s rRNA), and bacterial 16s rRNA. The PCR was performed using a LightCycler (Roche Diagnostics [Schweiz] AG, Rotkreuz, Switzerland). Primers and probes of HHV-1–8 and the PCR conditions have been described.[24] Specific primers for the virus were used with Accuprime Taq (Invitrogen, Carlsbad, CA). The products were subjected to 40 cycles of PCR amplification. Hybridization probes were then mixed with the PCR products. Subsequently, real-time PCR was performed only for the HHVs, with the genomic DNA detected by multiplex PCR. The real-time PCR was performed using Amplitaq Gold (Applied Biosystems, Foster City, CA) and the Real-Time PCR 7300 system (Applied Biosystems). All of the products obtained were subjected to 45 cycles of PCR amplification. The value of the viral copy number in the sample was considered to be significant when more than 50 copies/tube (5000 copies/ml) were observed.

Statistical Analysis

Statistical comparisons of the CD4/CD8 ratios between the groups were carried out by nonparametric analysis using the Mann–Whitney *U* test. Sensitivity and specificity were calculated for the CD4/CD8 ratio of vitreous and peripheral samples in detecting sarcoidosis. Positive predictive value was also estimated from sensitivity and specificity value:

$$\text{predictive value (\%)} = \text{pretestprobability} \times \text{sensitivity}/100/ [\text{pretest probability} \times \text{sensitivity}/100 + (100 - \text{pretest probability}) \times (100 - \text{specificity})/100]$$

Pretest probability was calculated as 17% according to the percentage of patients with ocular sarcoidosis in our uveitis outpatient clinic. Moreover, performances of CD4/CD8 ratio and its cutoff points were assessed using the receiver operator curve space. These analyses were performed using Prism software, version 5.0.1 (Graph Pad Software, San Diego, CA).

Results

Flow Cytometric Analysis of Vitreous Fluid and Peripheral Blood Samples

Flow cytometric data are summarized in Tables 1 and 2 (available at http://aaojournal.org). Representative flow cytometric data of sarcoid vitreous samples are shown in Figure 1 (sample obtained from a 59-year-old subject with confirmed ocular sarcoidosis; first line of Table 1, available at http://aaojournal.org). In the sarcoidosis group, the mean CD4/CD8 ratios of vitreous and peripheral blood sample were 40.7 (95% confidence interval [CI], 3.5–77.9) and 3.0 (95% CI, 2.5–3.5), respectively. In each classification of the ocular sarcoidosis group, the mean CD4/CD8 ratios of vitreous and peripheral blood sample were 70.0 (95% CI, −34.6 to 174.6) and 2.7 (95% CI, 1.9–3.5) for definite ocular sarcoidosis, 26.9 (95% CI, 15.4–38.4) and 3.1 (95% CI, 2.4–3.8) for presumed ocular sarcoidosis, and 22.9 (95% CI, 7.9–37.9) and 3.5 (95% CI, 2.5–4.5) for probable ocular sarcoidosis, respectively. In the nonsarcoidosis group, the mean CD4/CD8 ratios of vitreous and peripheral blood sample were 2.0 (95% CI, 1.1–2.9) and 2.0 (95% CI, 1.5–2.5), respectively. The CD4/CD8 ratios of lymphocytes obtained from the vitreous samples of patients with ocular sarcoidosis were significantly higher ($P < 0.0001$) than those in the vitreous of the nonsarcoidosis group (Fig 2A). Also, a significant difference was observed in the CD4/CD8 ratio of peripheral lymphocytes between the sarcoidosis and nonsarcoidosis groups ($P = 0.0163$) (Fig 2B). In addition, the CD4/CD8 ratios of lymphocytes obtained from the vitreous samples were significantly higher ($P < 0.0001$) than those of the peripheral blood samples in the ocular sarcoidosis group (Fig 2C). However, in the nonsarcoidosis group, no significant difference was found ($P = 0.67$) between the CD4/CD8 ratio of the vitreous samples and the peripheral samples (Fig 2D).

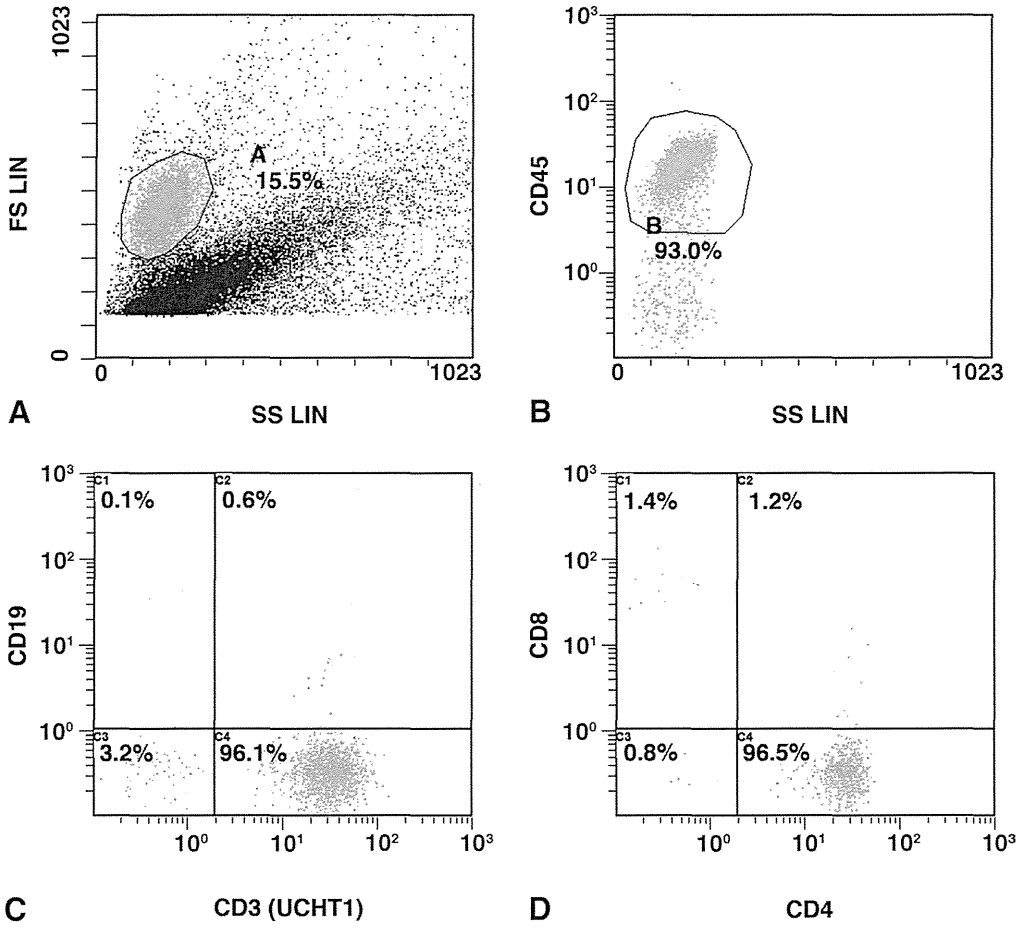

Figure 1. Representative flow cytometric data in confirmed ocular sarcoidosis. Total cells from vitreous samples (A) were gated with CD45+ cell population (B), CD3+ or CD16+CD56+ cell population (C), and CD4+ or CD8+ cell population (D). FS = forward scatter; LIN = liner; SS = side scatter.

Multiplex Polymerase Chain Reaction Analysis Results of Vitreous Samples

In the nonsarcoidosis group, multiplex PCR revealed varicella zoster virus DNA in 1 patient with an epiretinal membrane and herpes virus infection, bacterial 16s rRNA in 4 patients with endophthalmitis, and HSV-2 DNA in 3 patients with acute retinal necrosis and iritis (Table 2, available at http://aaojournal.org). Moreover, the PCR analysis detected cytomegalovirus in 2 patients. *Toxocara* DNA was not detected by PCR analysis, but the 2 positive results were elicited on *Toxocara* serology[26] using vitreous samples and peripheral blood. No viral or bacterial DNA was detected in the sarcoidosis group, patients with idiopathic uveitis, or patients with primary intraocular lymphoma.

Statistical Analysis

All of the patients in the ocular sarcoidosis group had high CD4/CD8 ratios (>3.5). Only 1 patient in the nonsarcoidosis group with endophthalmitis had a high CD4/CD8 ratio. The sensitivity and specificity of the vitreal CD4/CD8 ratio with a cutoff point of 3.5 were 100% and 96.3%, respectively, for the diagnosis of ocular sarcoidosis. The sensitivity and specificity of the peripheral CD4/CD8 ratio with a cutoff point of 2.7 were 52.6% and 84.6%, respectively, for the diagnosis of ocular sarcoidosis. The positive predictive values of vitreal and peripheral CD4/CD8 ratios were 84.2% and 41.2%, respectively. We compared the diagnostic performance of vitreal and peripheral CD4/CD8 ratio by plotting their performance in receiver operator curve space (Fig 3). The vitreal CD4/CD8 ratio was shown to have better diagnostic performance than the peripheral CD4/CD8 ratio by calculating the area under the curve. The best cutoff points for vitreal and peripheral CD4/CD8 ratio were 3.5 and 2.7, respectively.

In all patients involved in this study, the clinical course was favorable, with no adverse events resulting from the surgical procedures, including the collection of the vitreous samples.

Discussion

To the best of our knowledge, this is the first report to describe the unique immunologic features of vitreous lymphocytosis in ocular sarcoidosis characterized by a high CD4/CD8 ratio. A CD4/CD8 ratio of vitreous-infiltrating lymphocytes greater than 3.5 provided a diagnosis of ocular sarcoidosis with a sensitivity of 100% and a specificity of 96.3%, which are both remarkably high values. A CD4/CD8 ratio greater than 3.5 in lymphocytes obtained from BAL fluid had a sensitivity of 53% and a specificity of 94%.

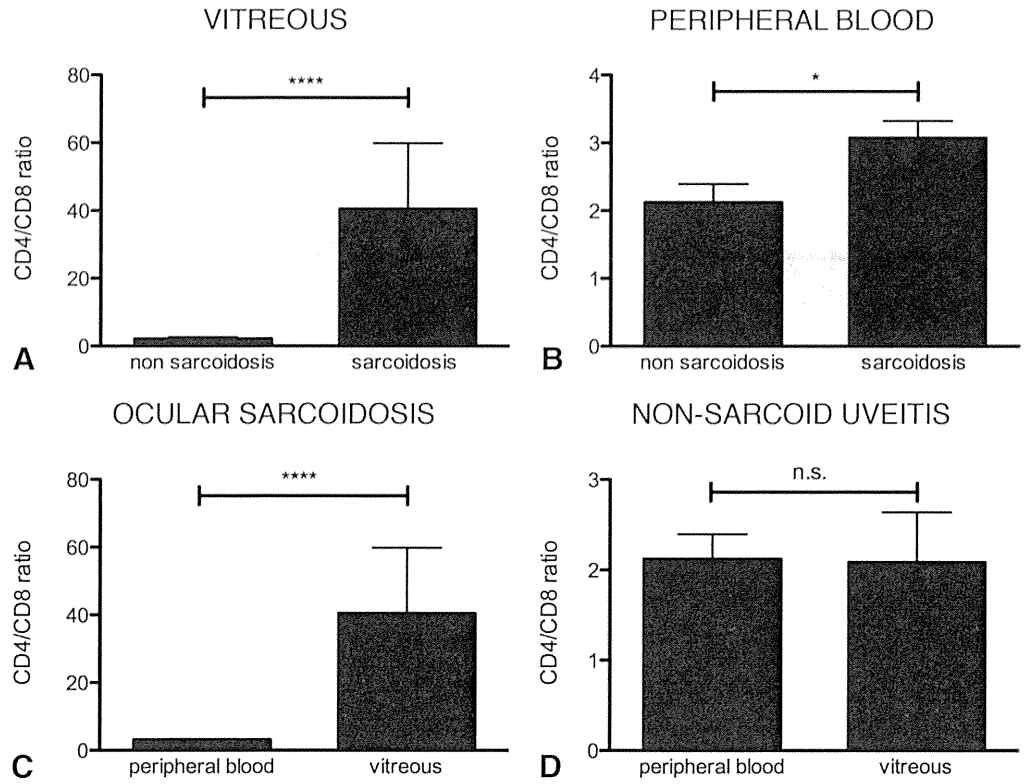

Figure 2. Quantitative analysis of CD4/CD8 ratios in vitreous and peripheral blood samples of sarcoidosis and nonsarcoidosis groups. A, Vitreous: T lymphocytes obtained from the ocular sarcoidosis vitreous samples were significantly higher (****$P < 0.0001$) than those of nonsarcoidosis vitreous samples. B, Peripheral blood: A significant difference was observed in the CD4/CD8 ratio of peripheral lymphocytes between the sarcoidosis and nonsarcoidosis groups (*$P = 0.0163$). C, Ocular sarcoidosis: CD4/CD8 ratio of T lymphocytes between the vitreous and peripheral blood samples in the sarcoidosis group (****$P < 0.0001$). D, Nonsarcoid uveitis: In the nonsarcoidosis group, there was no significant difference between the vitreal and peripheral blood CD4/CD8 ratios of T lymphocytes. n.s. = not significant.

Thus, our results indicate that a high CD4/CD8 ratio of lymphocytes obtained from the vitreous has a high diagnostic value, comparable to that of a high CD4/CD8 ratio in BAL fluid lymphocytes.

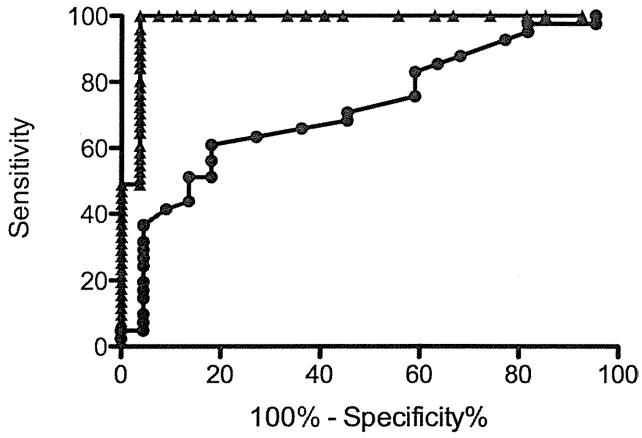

Figure 3. Performance of CD4/CD8 ratios in vitreous and peripheral blood samples discriminating sarcoidosis and nonsarcoidosis groups in receiver operator curve space. The comparison of diagnostic performance with vitreal and peripheral CD4/CD8 ratio by plotting their performance in receiver operator curve space.

Patients with sarcoidosis first present in the clinical setting with a variety of symptoms, and ocular symptoms account for a relatively high percentage. It has been reported that approximately 30% to 60% of patients with sarcoidosis manifest ocular involvement.[13–16] Kataoka[27] reported that ocular symptoms are the most frequently seen symptom at first presentation in Japanese patients. At the present time, diagnosis of sarcoidosis presenting with ocular manifestation requires other systemic features to confirm diagnosis. However, there are considerable numbers of suspected ocular sarcoidosis cases with a negative result in the systemic search.[28] Transbronchial lung biopsy and BAL are said to be relatively less-invasive procedures, yet there is currently disagreement as to whether these procedures should be performed in patients without clinical and radiologic pulmonary presentation. Ocular sarcoidosis manifests peculiar focal lymphocytosis characterized by an elevated CD4/CD8 ratio, and this unique CD4/CD8 pattern is significantly higher than that of peripheral blood. This immunologic dissociation between ocular and peripheral lymphocytes is an important aspect for the diagnosis of ocular sarcoidosis, because this feature is not observed in other types of uveitis. Therefore, it is rational to use this immunologic information to diagnose sarcoidosis with an active lesion localized in the eye to enable an early and less-invasive diagnosis. Of note,

the CD4/CD8 ratio in the peripheral blood was not so high in patients with sarcoidosis but still significantly different than in the patients without sarcoidosis. This may account for the systemic aspect of this disorder, showing that this immunologic feature is not only limited to the active lesion site.

Likewise, the hallmark of sarcoidosis in other organs is the identification of a noncaseating epithelioid cell granuloma. There are reports of identifying granulomata in conjunctiva[29] and the Schlemm's canal,[30] but presentation of granuloma in the conjunctiva is a rare case, and a Schlemm's-canal biopsy is only possible at the time of trabeculectomy surgery, which is only performed when sarcoidosis-induced glaucoma cannot be controlled by topical medication. Because it is rare to find a granuloma in the vitreous, and retinal biopsies are accompanied with the risk of serious complications such as retinal detachment, flow cytometric analysis of vitreous-infiltrating lymphocytes can be considered as a helpful and safer diagnostic tool.

Diagnostic vitrectomy for chronic uveitis of unknown cause is widely performed.[20,21] Davis et al[23] reported the usefulness of vitreous specimen analysis, including flow cytometry, for the differential diagnosis of intraocular lymphoma, chronic infectious endophthalmitis, and atypical chorioretinitis. Our results show that through cytometric analysis of a vitrectomy specimen, it is possible to distinguish sarcoidosis from other types of uveitis. Multiplex PCR is also helpful for ruling out other diagnoses, such as infection. There are reports that have suggested an association between sarcoidosis and propionibacterial infection.[6,30] There is no evident explanation among propionibacterial infection, noncaseating granuloma, and lymphocyte subsets at the present time, but it is safe to say that ocular sarcoidosis and pulmonary sarcoidosis have identical pathologic features. Further experiments are warranted to resolve this kind of discrepancy.

Basic treatment for ocular sarcoidosis involves corticosteroid medication, but vitrectomy for chronic uveitis-like sarcoidosis, especially for those cases resistant to pharmacologic treatment, is well documented in the published literature. Good outcomes of surgical treatment for sarcoidosis are reported for vitreous opacity resistant to medical treatment,[31] cystoid macular edema,[32] and epiretinal membrane.[33] Likewise, our operating procedures (including vitreous sample extraction) had no adverse events, indicating that this diagnostic procedure is safe for the patient. Clinical course was favorable for all the patients, and good visual acuity was achieved during the follow-up period (data not shown). Because of this good clinical outcome, we believe that a diagnostic vitrectomy should be recommended whenever a patient with suspected sarcoidosis has symptoms associated with vitreous opacity or epiretinal membrane.

In conclusion, in patients with systemic sarcoidosis (based on the international classification), diagnostic vitrectomy with flow cytometric lymphocyte analysis is useful as an adjunct to the standard diagnosis of ocular sarcoidosis. In these patients, information obtained from vitreous samples, especially a CD4/CD8 ratio greater than 3.5, has high diagnostic value, comparable to the CD4/CD8 ratio in BAL fluid lymphocytosis in pulmonary sarcoidosis. The role of diagnostic vitrectomy with CD4/CD8 analysis in subjects with questionable systemic diagnoses remains a topic for additional study.

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Ocular surface inflammation is regulated by innate immunity

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ABSTRACT

On the ocular surface, as in the intestines and airway, the surface epithelium serves a critical function as the front-line defense of the mucosal innate immune system. Although the detection of microbes is arguably the most important task of the immune system, an exaggerated epithelial host defense reaction to endogenous bacteria may initiate and perpetuate inflammatory mucosal responses.

In this review we first describe commensal bacteria found on the ocular surface, which is in contact with the ocular surface epithelium. We also discuss the innate immunity of the ocular surface epithelium and we present the allergic reaction regulated by ocular surface epithelial cells. We address ocular surface inflammation due to disordered innate immunity and we present our hypothesis that the onset of Stevens-Johnson syndrome (SJS) with severe ocular surface complications, a devastating ocular surface inflammatory disease, is strongly associated with abnormality of the innate immune system.

In this review we raise the possibility that some ocular surface inflammatory diseases are pathogenetically related with a disordered innate immune response.

Focusing on the innate immunity of the ocular surface might help to elucidate the pathogenesis of various ocular surface diseases.

Contents

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1. Introduction

On the ocular surface, as in the intestines and airway, the surface epithelium serves a critical function as the front-line defense of the mucosal innate immune system (Haynes et al., 1999; Streilein, 2003). Epithelial cells lining mucosal surfaces play a pivotal role in innate immunity; upon challenge they secrete chemokines and other immune mediators. The ocular surface epithelium also features all isoforms of human beta defensins and can produce inflammatory cytokines such as interleukin (IL)-1α, IL-1β, tumor necrosis factor (TNF)α, IL-6, and IL-8. Anti-microbial molecules such as IgA, lysozyme, and lactoferrin are found in tear fluids. Goblet cells in conjunctival epithelium produce mucin. Thus, the ocular surface possesses many non-specific defense mechanisms against microbes (Fig. 1).

Although the detection of microbes is arguably the most important task of the immune system, an exaggerated epithelial host defense reaction to endogenous bacteria may initiate and perpetuate inflammatory mucosal responses (Bouma and Strober, 2003; Strober, 2004; Strober et al., 2002).

In this review we first describe commensal bacteria found on the ocular surface, which is in contact with the ocular surface epithelium. We also discuss the innate immunity of the ocular surface epithelium and we present the allergic reaction regulated by ocular surface epithelial cells. We address ocular surface inflammation due to disordered innate immunity and we present our hypothesis that the onset of Stevens-Johnson syndrome (SJS) with severe ocular surface complications, a devastating ocular surface inflammatory disease, is strongly associated with abnormality of the innate immune system.

We propose that the pathogenesis of some human ocular surface inflammatory diseases is related to a disordered innate immune response.

2. Commensal bacteria on the ocular surface

2.1. Commensal bacteria

Bacterial flora comprised of gram-positive and gram-negative organisms can be found on the skin and in mucosal tissues. The ocular surface and other mucosal tissue are host to commensal bacteria (Doyle et al., 1995; Hara et al., 1997; Ueta et al., 2007a). To examine these organisms present on the ocular surface we harvested commensal bacteria from the lower conjunctival sacs of 42 healthy volunteers using CultureSwab (Becton Dickinson, Brescia, Italy) without touching the lids. *Staphylococcus epidermidis* (*S. epidermidis*) was isolated from 45% of the volunteers and *Propionibacterium acnes* (*P. acnes*) from 31% (Ueta et al., 2007a). Interestingly, although the ocular surface epithelium is in constant contact with bacteria and bacterial products, the healthy ocular surface is not in an inflammatory state. The ocular surface harbors unique innate immune mechanisms to regulate inflammation induced by microbes (Fig. 2).

2.2. Polyclonality of S. epidermidis

In humans, the predominant staphylococcus species *S. epidermidis* is widely distributed over the body surface (Kloos and Musselwhite, 1975). When we used pulsed-field gel electrophoresis (PFGE) to examine the diverse genetic background of *S. epidermidis* isolated from the ocular surface of healthy donors we found that the bacterium was polyclonal (Ueta et al., 2007a) (Fig. 3A).

For analysis, DNA bands were compared by visual inspection and interpreted according to Tenover et al. (1995). Based on the number of DNA fragments that exhibited different pulse patterns, strains with identical pulse patterns, and those with 2–3 or 4–6 fragments of different patterns were considered indistinguishable, closely related, and possibly related, respectively. When more than 6 DNA fragments manifested a different migration pattern, the isolates were considered to be unrelated.

We analyzed commensal bacteria isolated from the conjunctival sac, upper and lower lid margins, and upper and lower meibomian glands of another 40 healthy volunteers. *S. epidermidis* was isolated from 12 individuals; 7 harbored these bacteria at multiple ocular surface sites. Interestingly, *S. epidermidis* organisms isolated from multiple sites in single subjects were polyclonal. When we analyzed *S. epidermidis* isolated from the conjunctival sac of the same donor at different time points, we also found that the organisms were polyclonal and yielded multiple colonies. At some sampling points there was a change in the dominant strain (Ueta et al., 2007a).

Although *S. epidermidis* is a common component of the normal ocular flora, it can lead to chronic blepharitis, conjunctivitis, and keratitis, especially in immunocompromised hosts (Baum, 1978; Pinna et al., 1999), suggesting that opportunistic infection with *S. epidermidis* is reflective of the status of the host. We encountered one instance in which monoclonal *S. epidermidis* was isolated from multiple sites in both eyes (Fig. 3B). The host was an immunocompromised patient who had undergone bone marrow transplantation. Disruption of the balance between *S. epidermidis* and the immune status of this host may have resulted in the monoclonality of these bacteria. Based on these observations we postulated that a balance between commensal bacteria and the host mucosal immunity maintains the polyclonality of *S. epidermidis*, which may contribute to homeostasis of the commensal organisms, and that a weakened host mucosal immune status may contribute to their pathogenicity. When the host mucosal immunity is normal, commensal bacteria are in a symbiotic relationship with the host, however, if the host's mucosal immunity is abnormal, commensal bacteria can become pathogenic (Ueta et al., 2007a) (Fig. 3C).

The finding of Seal et al. (1985) that a specific strain of *S. epidermidis* could increase in the lids of blepharitis patients and manifest pathogenicity on the ocular surface may indicate that the role of *S. epidermidis* on the ocular surface requires further investigation.

2.3. Hypersensitivity to bacteria

Ocular surface inflammations such as catarrhal ulcers (marginal keratitis) and phlyctenular keratitis are thought to reflect

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