A Relationship Between Varicella-Zoster Virus–Specific Delayed Hypersensitivity and Varicella-Zoster Virus–Induced Anterior Uveitis

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Background: We recently reported that acute retinal necrosis in humans develops in a setting where delayed hypersensitivity (DH) to the varicella-zoster virus (VZV) antigen was absent, implying that virus-specific DH mitigates against acute retinal necrosis.

Objective: To determine whether a similar correlation exists for patients with anterior uveitis caused by VZV.

Design: Using VZV and purified protein derivative (PPD) antigens to evaluate DH, we skin tested patients with acute, VZV-induced anterior uveitis (herpes zoster ophthalmicus [ZO-AU]) (n=12), those with uveitis caused by VZV in the absence of dermatitis (zoster sine herpete [ZSH-AU]) (n=3), and age-matched patients whose ophthalmic herpes zoster was unassociated with uveitis as controls (n=7). Varicella-zoster virus–induced anterior uveitis was diagnosed by polymerase chain reaction methods and serum antibody titration. Serum samples were collected and analyzed for anti-VZV antibody titers. Anterior uveitis activity was assessed clinically. Delayed hypersensitivity skin tests were repeated in patients with zoster sine herpete 3 months after onset, when ocular recovery had taken place.

Results: All patients with VZV-induced skin disease alone (control group) displayed intense DH when tested with VZV and PPD antigens. By contrast, only 4 (33%) of 12 patients with ZO-AU had a positive DH to VZV, whereas 11 (91.6%) of these patients displayed positive PPD skin reactions. The clinical intensity of anterior uveitis correlated negatively with VZV DH responses (P<.05). Serum anti-VZV and anti–herpes simplex virus antibody titers were comparable in DH-positive VZV cases and in DH-negative patients with uveitis. Patients with uveitis and ZSH-AU also displayed absent VZV-specific DH, although their PPD responses were normal.

Main Outcome Measures: Varicella-zoster virus–specific DH, PPD-specific DH, VZV-specific antibody titration, and intraocular pressure in patients with ZO-AU.

Conclusions: Absence (or loss) of DH reactivity to VZV antigens seems to be a concomitant feature of VZV uveitis of high intensity, implying that virus-specific DH may interfere with the emergence of VZV-induced anterior uveitis, as it does for acute retinal necrosis.

Clinical Relevance: In a clinical setting, absence of virus-specific DH to anterior uveitis caused by VZV may not only reveal a possible pathogenic mechanism, but a negative DH response may prove useful in diagnosing ZSH-AU in the acute stage.

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thalamic division is the most frequently affected branch of the trigeminal nerve, and when VZV in this branch is reactivated, it can affect various ocular tissues, causing blepharitis, conjunctivitis, scleritis, keratitis, anterior uveitis, vitritis, and retinitis.

Recovering virus from aqueous humor aids in making the diagnosis of isolated herpetic iridocyclitis, and almost invariably, the uveitis is on the same side as the affected trigeminal branch. Uveitis caused by VZV is an extremely common manifestation of recurrent disease. In some cases, virus particles have been found in the anterior chamber and may contribute to the uveitis.

How VZV-induced uveitis suggests that the emergence of VZV-induced uveitis during acute VZV reactivation may be dependent on the attenuation of DH reactivity to VZV. A similar inverse correlation exists between VZV-induced acute retinal necrosis and virus-specific DH.

### PATIENTS AND METHODS

#### PATIENTS

Twelve patients with acute anterior uveitis with VZV-induced facial skin disease (herpes zoster ophthalmicus [ZO-AU] group; mean ± SD age: 49.6 ± 18.2 years) were selected from the patient population with uveitis at the Department of Ophthalmology, Tokyo Medical University Hospital from 1999 to 2001. Three patients with acute anterior uveitis caused by VZV without VZV-induced facial skin disease (zoster sine herpete [ZH-AU] group) were also selected. Seven age-matched patients with VZV-infection of the facial skin who displayed no uveitis (ophthalmic herpes zoster without uveitis group) were chosen as VZV-infected controls. In addition, 6 patients with Vogt-Koyanagi-Harada disease or HLA-B27–positive acute anterior uveitis were selected as noninfected controls.

#### SAMPLES

All cases of serum samples and some cases of aqueous humor in patients with anterior uveitis with VZV-like facial skin disease were collected at the first visit to the clinic. Similar samples were collected from patients with anterior uveitis without facial skin disease as a means to determine the presence of VZV infection. Informed consent was obtained from each patient before skin test assays were performed.

### POLYMERASE CHAIN REACTION

For the diagnosis of anterior uveitis caused by VZV, polymerase chain reaction (PCR) methods were performed by the technique described by Saiki et al., Usui et al., and Kezuka et al.

### ASSAY OF ANTI-VZV ANTIBODY TITER IN SERUM SAMPLES

For help with the diagnosis of anterior uveitis caused by VZV, viral antibody titer of serum samples were determined by the fluorescent antibody technique.

#### SKIN TEST ASSAY OF DH

At their first visit to the clinic, and before systemic steroid therapy and an antiviral agent regimen were instituted, 14 patients with ZO-AU and ZH-AU, as controls, 8 age-matched patients with VZV infection of the facial skin who displayed no uveitis, and 5 patients with Vogt-Koyanagi-Harada disease or HLA-B27–positive acute anterior uveitis, were skin tested with 0.1 mL of VZV antigen (Tanabe Co, Osaka, Japan) and purified protein derivative (PPD) (Takeda Co, Osaka) antigens. Delayed hypersensitivity reactions were evaluated 24 and 48 hours after the first visit to the clinic. Skin tests were performed using the technique described by Kezuka et al. In detail, we used varicella virus of Kawaguchi strain for the preparation of skin antigen. The test antigen preparation includes VZV glycoproteins (gp 3 and gp 5) (80-100 µg/mL). We used PPD tuberculin (0.5 µg/mL), an antigen derived from the tubercle bacillus, as the positive control antigen for skin tests. Positive responses were characterized by cutaneous erythema at the injection sites, measuring greater than 5 mm in diameter at 24 hours and 48 hours for VZV antigen; and greater than 10 mm in diameter at 48 hours for PPD antigen. In some patients, the VZV skin test was repeated 3 months after the initial onset of intraocular disease.

#### CLINICAL EVALUATION OF ANTERIOR UVEITIS CAUSED BY VZV

At the first visit to the clinic, all patients with anterior uveitis caused by VZV were divided into a “2+” (ie, severe) group and a “1+” (ie, mild) group. The severity of disease in these patients proved to be greatest on the day of their initial clinic visit, which typically occurred a few days (or less) after the onset of disease. The observers were 2 of us (T.K. and J.S.), who each evaluated anterior chamber cells and flare using the modified grading system described by Hogan et al. In fact, Hogan and colleagues proposed the grading system of evaluation of uveitis that used an approximately 1 x 1-mm slit beam. They graded anterior chamber cells and flare on a scale of 0 to 4+. We modified our system as follows: 1+ indicates “mild uveitis” (equivalent to their grades 1+ and 2+) and 2+ indicates “severe uveitis” (equivalent to their grades 3+ and 4+). In specific detail, using a 1 x 1-mm slit beam, we considered 1+ as “mild uveitis,” with 5 to 20 cells in the anterior chamber, moderate anterior chamber flare (iris and lens images are clear), and no large keratic precipitates; we considered 2+ as “severe uveitis,” with 20 cells in the anterior chamber, marked flare (iris and lens images are hazy) in the anterior chamber, and large keratic precipitates on the corneal endothelium. No patients had received topical treatment prior to their first visit to our clinic, and treatment with topical corticosteroids was administered after the first medical examination. No patients had a history of corticosteroid-induced elevation of intraocular pressure (IOP). Within 2 weeks after the first visit, the IOP of patients with VZV-induced acute anterior uveitis was measured. Intraocular pressure values in excess of 21 mm Hg were considered diagnostic of secondary glaucoma.

Differences between groups to be compared were analyzed by Mann-Whitney U test; P < .05 was considered to be significant.

#### RESULTS

### CHARACTERISTICS OF PATIENTS WITH VZV-AU AND THEIR CONTROLS

Fourteen patients selected from a clinical population with uveitis were selected for study (Table 1). These pa-
tients were diagnosed with uveitis caused by VZV and included patients from the ZSH group. Within this group, the uveitis of 8 patients was categorized as severe, while that of 4 others was categorized as mild. Based on history, these patients first visited the clinic within 2 to 37 days after the onset of skin disease, except for those in the ZSH-AU group. Serum samples were collected during the acute phase of the disease. The formal diagnosis of VZV-AU was established by the titer of anti-VZV antibody, and in cases of patients with ZSH, the diagnosis was established by PCR analysis using the aqueous humor (data not presented).

VZV-SPECIFIC DH IN PATIENTS WITH ZO-AU

To investigate the relationship between cell-mediated immune responses to the VZV antigen and the presence of ZO-AU, we performed skin testing for patients with VZV-AU and ophthalmic herpes zoster without uveitis with VZV antigens. The results of this study are presented in Table 2. There was no significant difference between 24-hour and 48-hour erythema responses in any of the subjects. All 7 patients in the control group displayed intense DH when tested with VZV antigen. By contrast, only 1 (12.5%) of 8 patients with severe ZO-AU displayed a positive VZV skin test. Moreover, 3 (75.0%) of 4 patients with mild ZO-AU displayed a positive VZV skin test. The clinical intensity of anterior uveitis correlated negatively with VZV DH responses (P < .05), and the difference between DH responses in patients with ZO-AU as compared with patients with ophthalmic herpes zoster without uveitis is statistically significant (P < .001).

Thus, patients with ZO-AU have a very high probability of displaying no virus-specific DH at the time of diagnosis, unlike in VZV-infected patients without uveitis, whose DH to VZV antigens is uniformly intense.

COMPARISON OF DH TO VZV AND PPD IN PATIENTS WITH ZO-AU

The impaired DH to VZV antigens demonstrated above can be explained in 2 ways. (1) Patients with ZO-AU suffer from a global loss of DH reactivity to many (or all) antigens; and (2) patients with ZO-AU suffer from a selective loss of DH reactivity to VZV viral antigens, but not to other antigens. To discriminate between these possibilities, each patient was also skin tested with PPD, an antigenic preparation derived from the tubercle bacillus...
Acute retinal necrosis caused by VZV correlates positively with an elevation of serum anti-VZV antibody titers. We have previously reported that the magnitude of the anti-VZV antibody response in these patients is inversely proportional to their ability to display VZV-specific DH. We wondered if patients with ZO-AU with impaired VZV-specific DH might resemble patients with acute retinal necrosis in this manner. We analyzed the titers of anti-VZV and anti-herpes simplex virus anti-bodies in serum samples from patients with ZO-AU using a fluorescent antibody technique. Serum samples collected from patients with VZV skin disease without uveitis served as controls. As the data presented in Table 1 and Table 4 reveal, anti-VZV and anti-herpes simplex virus serum antibody titers were comparable in DH-positive and DH-negative patients with ZO-AU. This result indicates that ZO-AU is associated with elevated serum anti-VZV antibody titers, and that the magnitude of the antibody titer is not related to the ability of these patients to display VZV-specific DH.

**COMPARISON OF IOP ELEVATION IN PATIENTS WITH ZO-AU**

The results presented thus far can be interpreted to mean that the presence of DH directed at VZV antigens protects against the development of severe ZO-AU in patients infected with the virus. Elevated IOP is a characteristic feature of acute ZO-AU. Using this as an indicator of the presence of AU, we measured the IOP of patients with ZO-AU and compared their levels with the presence or absence of virus-specific DH. The results are displayed in Table 5. The average IOP detected among patients with ZO-AU who had negative DH responses was 27.4 ± 6.4 mm Hg, whereas the average IOP detected among patients with ZO-AU who had positive DH was 20.3 ± 5.7 mm Hg (P < .05). This comparison suggests that the absence of a positive VZV DH skin test in a patient with uveitis may herald the development of severe ZO-AU.

**VZV-SPECIFIC DH IN PATIENTS WITH ZSH**

To test the validity of this suggestion, we examined VZV DH responses in patients with VZV without dermatitis (ZSH). In our experience, diagnosing uveitis caused by VZV in the absence of an attendant dermatitis is difficult. Along with VZV skin testing for patients with ZSH, we also skin tested patients with Vogt-Koyanagi-Harada disease or HLA-B27–positive acute anterior uveitis as controls. We skin tested 3 patients with ZSH, in whom VZV DNA was detected in aqueous humor samples using the PCR (data not presented). As presented in Table 6, these patients with ZSH failed to display VZV-specific DH, whereas 5 (83%) of 6 patients with Vogt-Koyanagi-Harada disease or HLA-B27–positive acute anterior uveitis responded to VZV antigens. As anticipated, PPD responses of patients with ZSH were normal (Table 1).
Approximately 40% of patients who develop ophthalmic herpes zoster (ie, reactivation of infection of the ophthalmic branch of the trigeminal nerve with VZV) develop anterior uveitis. Individuals with involvement of the external nasal nerve that supplies the side of the nose are at particular risk (Hutchinson symptom). In the present study, we observed that patients with ophthalmic herpes zoster without uveitis displayed intense VZV-specific DH, whereas patients with a similar infection complicated by the presence of anterior uveitis displayed no VZV-specific DH. At the simplest level, these results suggest that an important factor in determining whether a VZV infection of the ophthalmic branch of the trigeminal nerve will proceed to uveitis is whether the patient retains a high level of VZV DH reactivity. This curious result is strongly reminiscent of our previously reported finding that patients who develop VZV acute retinal necrosis are selectively and transiently deficient in virus-specific DH reactivity.1 Together, these findings imply the absence of virus-specific DH in the pathogenesis of intraocular disease caused by VZV. The corollary of this implication is that preservation of virus-specific DH at the time of a VZV infection is an important barrier to the development of intraocular infection with the virus.

Not only is acute uveitis itself a serious clinical problem in patients with ophthalmic herpes zoster, but patients with ZO-AU are at a significant risk of developing iris atrophy (20%), secondary glaucoma (10%), and secondary cataracts.10 Of the patients with ZO-AU who we enrolled in this study, 80% displayed elevated IOP compatible with secondary glaucoma. This was especially true of patients with deficient VZV-specific DH. Similarly, iris atrophy in our patients tended to be observed in patients with impaired VZV DH (Table 1). Together, these findings suggest that patients with impaired VZV DH are at a higher risk of developing the secondary complications of ZO-AU, and that once again, VZV DH functions as a barrier to the emergence of these secondary complications.

Varicella-zoster virus typically infects humans via the skin. A large majority of the Japanese population displays positive DH responses to the VZV antigen by age 12 years because of spontaneous contraction of chickenpox. Those who have not had a chickenpox infection by this time are offered the VZV vaccination, and the vast majority of these individuals receive the vaccination. This accounts for the observation that DH responses to VZV among the Japanese are pervasive. A minority of adult Japanese people develop recurrent VZV infection, which appears clinically as a vesicular rash that localizes to a dermatomal distribution. The ophthalmic division of the trigeminal nerve is the most frequently affected facial dermatome except in rare cases of herpes zoster sine eruption (ZSH).11-13 Usually, the diagnosis of ZSH is based on a PCR assay of aqueous humor,14-17 but justification of paracentesis of the anterior chamber to obtain aqueous humor is sometimes tenuous. In an indirect way, our findings—that the VZV DH responses of 2 patients with ZSH were negative during acute infection, but were restored to positivity when the disease had been inactive—suggest that the skin test might be diagnostically useful in this difficult clinical circumstance. That is to say, a negative VZV DH skin test in a patient with suspected ZSH would tend to confirm the accuracy of the diagnosis. By contrast, a positive VZV DH skin test in this clinical setting would argue against a VZV infection of the anterior uvea. Since DNA amplification with PCR technology tends to be most successful at the early stages of intraocular infection, and since the VZV skin test is more convenient than anterior chamber paracentesis, we think it worthwhile to further study the predictability of negative or positive VZV DH responses in this situation.

Quite some time ago, Tanaka et al18 reported the results of their study of VZV-specific DH reactivity in 12 patients with ophthalmic herpes zoster. On the one hand, only 1 of their 12 patients with this condition had a positive skin test reaction within 2 weeks of the onset of the eruption, suggesting that cellular immunity to VZV antigens was impaired during the development of ophthalmic herpes zoster. On the other hand, they reported that all patients with ophthalmic herpes zoster displayed positive skin test results when tested 3 weeks after the appearance of the cutaneous manifestations of the disease. Unfortunately, the authors of that report failed to mention the severity of ZO-AU in their patients. Nonetheless, that report strongly resembles our current findings on patients with ZSH, as well as our previous report on patients with acute retinal necrosis. In both instances, DH reactivity to VZV antigens was impaired at the time of the onset of intraocular infection with VZV, yet the impairment proved to be transient. To explain these results, we have previously proposed that idiopathic reactivation of VZV in the anterior segment of an eye might promote transient suppression of DH, thereby silencing virus-specific CD4+ T cells that are required to prevent VZV infection of intraocular tissues.

Herpes zoster ophthalmicus and ZSH-AU are inflammatory ocular diseases restricted to the anterior segment of the eye, whereas VZV-induced acute retinal inflammatory ocular diseases restricted to the anterior segment of the eye.
necrosis is a retinal infection. In the mouse system, BALB/c mice that receive an anterior chamber injection of an antigen acquire an unusual systematic immune response, termed “anterior chamber–associated immune deviation” (ACAID).19,20 In this system, impaired antigen-specific DH coexists with high serum titers of antigen-specific antibodies. The results presented in this article serve to support our hypothesis that anterior chamber–associated immune deviation may be the immunologic mechanism that is triggered in the eyes of some patients undergoing idiopathic reactivation of VZV in the trigeminal ganglion. For reasons yet to be revealed, zosteriform spread of antigenic VZV particles to the anterior chamber leads to suppression of virus-specific T cells that mediate DH, and by so doing, rob the eye of the protection afforded by these CD4+ T cells. Acute viral infection of sensitive intraocular tissues ranging from iris to retina is the inevitable consequence. If we could understand how to abort anterior chamber–associated immune deviation in this situation, intraocular complications of ophthalmic herpes zoster might be eliminated from clinical ophthalmology.

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REFERENCE


