Evidence for Antigen-Specific Immune Deviation in Patients With Acute Retinal Necrosis

Takeshi Kezuka, MD, PhD; Jun-ich Sakai, MD, PhD; Norio Usui, MD, PhD; J. Wayne Streilein, MD; Masahiko Usui, MD, PhD

Background: Because experimental acute retinal necrosis (ARN) induced by herpes simplex virus in mice develops only if mice fail to acquire virus-specific delayed hypersensitivity (DH), although they produce antiviral antibodies (ie, anterior chamber–associated immune deviation), we sought to determine whether a similar inverse correlation exists for patients with varicella-zoster virus (VZV)–induced ARN.

Design: Patients with acute, VZV-induced ARN and age-matched control subjects were skin tested with VZV and purified protein derivative antigens to evaluate DH. Varicella-zoster virus–induced ARN was diagnosed using polymerase chain reaction and intraocular antibody quotient. Serum samples were collected and analyzed for anti-VZV and anti–herpes simplex virus antibody titers. Acute retinal necrosis activity was assessed clinically, and DH skin tests were repeated 3 months after onset when ocular recovery had taken place.

Results: Whereas controls displayed intense DH when tested with VZV and purified protein derivative antigens, a subset of patients with ARN displayed absent VZV-specific DH (although their purified protein derivative responses were normal). Patients with the most severe ARN had the lowest DH responses to VZV antigens. Serum anti-VZV antibody titers were higher in patients with ARN than in controls, and antiviral titer correlated inversely with the intensity of anti-VZV DH responses. Varicella-zoster virus–specific DH responses were restored in patients who recovered from ARN.

Conclusion: Varicella-zoster virus–ARN develops in a setting where DH reactivity to viral antigens is absent, implying that virus-specific DH might ameliorate the severity of ARN.

Clinical Relevance: Linking virus-specific DH to vulnerability to ARN in individuals infected with VZV might reveal an underappreciated pathogenic mechanism.


A CUTE RETINAL necrosis (ARN, Kirisawa-Urayama uveitis) is a destructive retinal disease in which an acute and rapidly progressing retinal inflammation leads to severe impairment of vision within days of onset.1-4 The clinical expression of this disease is usually limited to the eye, and the clinical features within the affected eye include acute peripheral necrotizing retinitis, retinal arteritis, vitritis, and panuveitis. As the inflammatory disease progresses it can promote retinal detachment that causes further visual deterioration.5 It is generally believed that ARN arises in the setting of an ocular infection with a member of the family of herpes viridae, such as varicella-zoster virus (VZV) or herpes simplex virus (HSV),5-8 but the precise pathogenesis of the retinal necrosis is still in doubt.

A similar intraocular inflammatory process that leads to ARN has been produced in laboratory animals.5-20 In this animal model system, the pathogenesis of ARN is linked to a deviant systemic immune response to the virus. To determine whether a similar circumstance might occur in humans with ARN, we studied the immune responses of a group of patients with acute disease in which reactivated VZV was found in the affected eye. Our results indicate that a high proportion of patients with ARN associated with VZV displayed a transient loss of virus-specific delayed hypersensitivity (DH), but their serum samples contained high titers of anti-VZV antibodies. On resolution of the intraocular inflammation, virus-specific DH recurred in most of these individuals.

RESULTS

DESCRIPTION OF PATIENTS WITH ARN ASSOCIATED WITH INTRAOCULAR VZV

Twenty-three patients, selected from a uveitis clinical population, participated in this study. These patients were diagnosed as

From the Department of Ophthalmology, Tokyo Medical University, Tokyo, Japan (Drs Kezuka, Sakai, N. Usui, and M. Usui); and Schepens Eye Research Institute and the Department of Ophthalmology, Harvard Medical School, Boston, Mass (Dr Streilein).
PATIENTS AND METHODS

PATIENTS

Twenty-three patients with ARN (mean ± SD age, 50.4 ± 8.8 years) were selected from the uveitis clinic population of the Department of Ophthalmology, Tokyo Medical University Hospital, Tokyo, Japan, from 1989 to 1999. Thirteen healthy persons, 12 patients with noninfectious uveitis (without sarcoidosis), and 7 patients with VZV infection of the skin who displayed no uveitis were also selected as control subjects.

SAMPLES

Aqueous humor and serum samples from patients with ARN were collected at the first visit to our office. Vitreous samples from patients with ARN were collected at the time of vitrectomy. Informed consent was obtained from each patient before skin test assay and collection of blood.

POLYMERASE CHAIN REACTION

For the diagnosis of ARN, polymerase chain reaction (PCR) methods were performed using the technique described by Saiki et al21 and Usui et al.22 In brief, a 25-µL aliquot of each sample was mixed with 25 µL of detergent buffer (potassium chloride, 50 mmol/L; Tris hydrochloride, 10 mmol/L [pH 8.3]; magnesium chloride, 1.5 mmol/L; gelatin, 0.1 mg/mL; 0.45% NP40; 0.45% Tween 20; and protease K, 0.06 mg/mL). Each sample was incubated at 60°C for 60 minutes and then reincubated at 95°C for 10 minutes to inactive protease K. Fifty microliters of PCR mixture was added to the samples after the incubations (final concentration: potassium chloride, 50 mmol/L; Tris hydrochloride, 10 mmol/L [pH 8.3]; magnesium chloride, 1.5 mmol/L; gelatin, 0.1 mg/mL; 0.2 µmol/L of the primer; 200 µmol/L of deoxynucleotriphosphates; and 2.5 U of Taq polymerase). Target sequence of the primer used in the PCR mixture was DNA segment from VZV (EcoRI D fragment, primer: 5′-TTACGCCAACGTTGCACAT CAAAAA-3′ and 5′-GAGCGCTTAACGGGAAGTAC-3′). Amplification was performed as follows: 1 minute at 94°C for denaturation, 2 minutes at 53°C for annealing, and 2 minutes at 72°C for extension. Thirty-five cycles were performed, and the 72°C step was extended to 6 minutes in the final cycle. The PCR products were visualized with UV light as a single band by staining with ethidium bromide after agarose gel electrophoresis (2.5% NuSieve/Seakem agarose gel).

ASSAY OF ANTI-VZV ANTIBODY TITERS IN SERA AND INTRAOCULAR FLUIDS

For help with the diagnosis of ARN, viral antibody titers of intraocular fluid (IOF) (aqueous humor and vitreous fluid) and serum samples were determined by the fluorescent antibody technique. Paired IOF and serum samples were tested at the same time. The antibody quotient was calculated from the total IgG levels in IOF and serum samples as follows: antibody quotient = (VZV-specific IgG titers in IOF/total IgG levels in IOF)/(VZV-IgG titers in sera/total IgG levels in sera). A coefficient of 6 or greater is considered diagnostic.

SKIN TEST ASSAY OF DH

At their first visit to the clinic, and before systemic corticosteroid therapy was instituted, 23 patients with acute, VZV-induced ARN and, as controls, 13 age-matched healthy subjects and 7 age-matched patients with VZV infection of the skin who displayed no uveitis were skin tested with 0.1 mL of VZV (Tanabe Co, Osaka, Japan)23-25 and purified protein derivative (PPD) (Takeda Co, Osaka) antigens to evaluate DH for 24 and 48 hours at the first visit to our office. 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, as the positive control antigen for skin tests. Positive responses were characterized by cutaneous erythema at the injection sites that measured 1 greater than 5 mm in diameter at 24 and 48 hours for VZV antigen23-25 and 2 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 ARN

At the first visit to the clinic, patients with ARN were divided into severe and mild groups. In the severe group, the affected eyes displayed advanced damage to the vascular arcade of the retina. In the mild group, the affected eye displayed damage that was only observable in the peripheral retina (Table 1).

STATISTICAL EVALUATIONS

Differences between groups to be compared were analyzed using the Mann-Whitney test; P<.05 was considered statistically significant.
The results of this study are presented in Table 1. The erythema response at the injection site was measured at 24 and 48 hours. Varicella-zoster virus antigen was injected intradermally into the skin of the forearm. The erythema response to VZV and VZV-ARN, we skin tested patients with VZV-ARN and controls with VZV antigens. For the possible relationship between cell-mediated immune response to VZV and VZV-ARN, we skin tested patients with VZV-ARN and controls with VZV antigen. For the second group of VZV-ARN, defined as having VZV sequences and a significant anti-VZV IgG quotient in ocular fluids.

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

For the possible relationship between cell-mediated immune response to VZV and VZV-ARN, we skin tested patients with VZV-ARN and controls with VZV antigen. Varicella-zoster virus antigen was injected intradermally into the skin of the forearm. The erythema response at the injection site was measured at 24 and 48 hours. The results of this study are presented in Table 2. There was no significant difference between 24- and 48-hour erythema responses in any of the subjects. As anticipated, all control subjects displayed a positive VZV skin test response. By contrast, less than 50% of patients with VZV-ARN displayed a positive VZV skin test response. This difference is highly significant (P<.001). Two (9%) of 23 patients with ARN who had bilateral disease, and both displayed negative VZV skin test and positive PPD skin test responses (data not shown). Thus, at least with respect to VZV-specific DH, patients with VZV-ARN can be classified into 2 groups: one contains patients with a positive VZV skin test response and the other contains those with a negative VZV skin test response.

**TUBERCULIN-SPECIFIC DH IN PATIENTS WITH ARN**

The absence of VZV-specific DH in the second group of patients with VZV-ARN can be explained in 2 ways. First, some patients with VZV-ARN might lack the ability to display DH to any antigen. Second, some patients with VZV-ARN might have a selective deficit in DH directed at VZV antigens but not to other antigens. To distinguish between these 2 possibilities, each patient was also skin tested with PPD, an antigen derived from the tubercle bacillus. In Japan, vaccination with bacille Calmette-Guérin is common, and as a consequence a high proportion of healthy individuals possess DH directed at PPD. The results of this study of PPD reactivity for patients with VZV-ARN are presented in Table 3. Seventy-eight percent of patients with VZV-ARN displayed positive PPD skin test responses. This proportion of PPD-positive subjects is similar to, albeit slightly lower than, that observed in the healthy population (approximately 95% are PPD positive). Among patients with VZV-ARN who had a positive skin test response to VZV antigens, 8 of 9 displayed reactivity to PPD. Similarly, among patients with VZV-ARN who had no response to VZV antigens, 10 of 14 displayed reactivity to PPD.

**Table 1. Criteria for Severity of VZV-ARN**

<table>
<thead>
<tr>
<th>Anterior Segment Inflammation</th>
<th>Vitritis</th>
<th>Vasculitis</th>
<th>Rate of Progression of Retinitis (Quadrants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe ARN</td>
<td>++</td>
<td>++</td>
<td>4‡</td>
</tr>
<tr>
<td>Mild ARN</td>
<td>+</td>
<td>+</td>
<td>1-3‡</td>
</tr>
</tbody>
</table>

*VZV indicates varicella-zoster virus; ARN, acute retinal necrosis. ‡Affected eyes displayed advanced damage to the vascular arcade of the retina (360° of the retina). †Affected eyes displayed damage that was observable only in the 90° to 270° peripheral retina.

**Table 2. DH Responses Elicited by VZV Antigen in Patients With VZV-ARN**

<table>
<thead>
<tr>
<th>Patients</th>
<th>DH−†</th>
<th>DH+‡</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>VZV-ARN</td>
<td>14 (61)</td>
<td>9 (39)</td>
<td>23</td>
</tr>
<tr>
<td>Skin disease with VZV infection (without uveitis)</td>
<td>0§</td>
<td>7 (100)</td>
<td>7</td>
</tr>
<tr>
<td>Healthy person</td>
<td>0</td>
<td>13 (100)</td>
<td>13</td>
</tr>
</tbody>
</table>

†Negative DH to VZV antigen. ‡Positive DH to VZV antigen. §P<.001, VZV-ARN vs skin disease with VZV infection. ||P<.001, VZV-ARN vs healthy person.

**Figure 1. Detection of varicella-zoster virus gene sequences by polymerase chain reaction.** The typical amplified products of polymerase chain reaction from aqueous humor and vitreous humor are separated in a 2.5% agarose gel and visualized by ethidium bromide staining. Lanes 1 to 4 are products from varicella-zoster virus genomes. N indicates negative control; P, positive control; and bp, base pair.
is inversely proportional to the severity of ARN. As displayed in Table 4, anti-VZV serum antibody titers of patients with VZV-ARN in DH− (DH-negative group) were significantly higher than those in patients with VZV-ARN in DH+ (DH-positive group) (P = .03). Moreover, anti-VZV antibody titers in serum samples from both groups of patients with VZV-ARN were higher than those in noninfectious uveitis controls (P = .007). We also measured the anti-HSV serum antibody titer of patients with VZV-ARN. The results were comparable for patients who were between DH− and DH+ (data not shown). These results indicate, first, that VZV-ARN is associated with an elevation of serum anti-VZV antibody titers and, second, that the magnitude of the antibody response is inversely proportional to the ability of the patient with VZV-ARN to display VZV-specific DH.

### COMPARISON OF VIRUS-SPECIFIC DH AND CLINICAL SEVERITY OF VZV-ARN

In the mouse model of ARN, DH to virus-specific antigens is inversely proportional to the severity of ARN. As displayed in Table 5, we compared the clinical severity of VZV-ARN with the presence or absence of virus-specific DH. Most patients with DH reactivity to VZV antigens displayed mild VZV-ARN. By contrast, most patients who lacked VZV-specific DH displayed severe ARN (P = .053). These results provide circumstantial evidence to support the contention that the presence of DH directed at VZV antigens protects against the development of severe ARN.

### DETECTION OF VZV-SPECIFIC DH IN PATIENTS WITH VZV-ARN DURING RECOVERY

In our clinical experience, ARN generally runs its course within 3 months of onset of the disease. Eleven patients with VZV-ARN and a negative VZV-specific DH response at 1 week were skin tested again with VZV antigens 3 months after disease onset. Clinical examination of the affected eyes at this time revealed little evidence of ongoing inflammation. Two patients with VZV-ARN whose DH reactivity was positive during acute disease were also tested at 3 months. The recovery phase results are presented in Figure 2 in comparison with DH results obtained at 1 week (during acute disease). With one exception, all of the subjects displayed positive skin test responses to VZV antigens (P < .001). These results suggest that the absence of virus-specific DH during acute ocular inflammation in a group of patients with VZV-ARN is transient.

### COMMENT

Although the precise pathogenesis of ARN is incompletely understood, there is convincing evidence that active replication by a herpes virus in the affected eye (especially in the retina) is an essential element. Because ARN typically occurs in individuals usually considered to be immune competent, it is fair to question the role of the immune system in protecting against this disease. Rochat et al performed DH skin tests to 7 common antigens (Candida, diphtheria, PPD, Proteus, Streptococcus, tetanus toxoid, and Trichophyton) in 9 patients with the clinical diagnosis of ARN. They reported that 4 of 9 patients were “anergic,” ie, lacked positive skin test responses to any of the test antigens. In addition, flow cytometric analysis of
peripheral blood leukocytes revealed an excessively high number of B lymphocytes. These results led the authors to suggest that a non–antigen-specific imbalance of the systemic immune system (impaired cellular immunity with intact humoral immunity) might be involved in ARN pathogenesis. Our study addressed these same points, but our results led us to a somewhat different conclusion. Although we skin tested our subjects with only one nonviral antigen (PPD) as a test of their global immune competence, the high incidence of positive DH to PPD in our patients with VZV-ARN indicated that as a group they were not anergic and that their systemic immune response seemed to be intact. Instead, our findings point to a virus antigen-specific aberration in the immune response of at least some patients with VZV-ARN.

We skin tested our patients with ARN shortly after the clinical diagnosis was made and before corticosteroid therapy was initiated. It is pertinent that most of the Japanese population acquires positive DH responses to VZV antigens by age 12 years. The timing of skin tests with respect to the clinical stage of ARN and the presence or absence of corticosteroid treatment were not described in the study by Rochat et al. Almost all of our patients with ARN who initially had a negative VZV skin test response reacquired this reactivity after 3 months. This indicates that the ability of such patients to display DH is volatile and that a negative response at one time might change to positive through time. Therefore, one explanation for the difference between our findings and those of Rochat et al might reside in the timing of skin tests and treatment with corticosteroids. Nonetheless, we found no evidence of significant impairment of DH directed at a nonviral antigen (PPD) at the time of disease onset or thereafter. Based on our results, we conclude that the loss of DH in patients with VZV-ARN is restricted to VZV antigens.

The virus-specific immune features of some of our patients with VZV-ARN closely resemble those of BALB/c mice that develop ARN after injection of HSV-1 into the anterior chamber of the fellow eye. The systemic deficit in DH was limited to VZV antigens (not PPD). In mice with ARN, only DH to HSV antigens is impaired. Absence of VZV-specific DH correlated with a very high serum titer of anti-VZV antibodies. In mice with HSV-induced ARN, high titers of anti-HSV antibodies are found in the serum. But VZV-ARN in humans and HSV-ARN in mice are not identical. For example, patients with VZV-ARN who lack DH during acute disease reacquire virus-specific DH as the disease resolves. By contrast, mice with HSV-ARN continue to lack virus-specific DH for at least 7 weeks after the disease subsides. More important, mice that acquire virus-specific DH after anterior chamber injection of HSV-1 never develop ARN in the contralateral eye, whereas a significant proportion of our patients with acute VZV-ARN displayed strong VZV-specific DH. The similarities and differences between human and mouse ARN probably mean that the mouse disease is an imperfect model system for the human disease. In addition, the differences in DH reactivity and anti-VZV antibody titers observed in our patients with VZV-ARN might reflect heterogeneity in the pathogenesis of human ARN.

The mouse model system of ARN most closely resembles the patients with VZV-ARN we assigned to DH–: absence of DH, high titers of serum anti-VZV antibodies. From what has been learned about the immune contribution to ARN in mice, we can speculate on the role of the immune system in the pathogenesis of this form of VZV-ARN in humans. Absence of the virus-specific CD4+ T cells that mediate DH robs the individual of the ability to prevent immunologically the spread of virus within the nervous system from the site of entry. Moreover, this unique pathway of viral migration has been linked to the type of antiviral immune response generated in recipient mice. BALB/c mice that receive an anterior chamber injection of HSV-1 (KOS strain) acquire an unusual systemic immune response, termed anterior chamber–associated immune deviation (ACAID). In this response, there is, on the one hand, a selective impairment of virus antigen-specific DH, whereas, on the other hand, there is a high serum titer of anti-HSV antibodies. Circumstantial evidence suggests that the lack of virus-specific DH in these mice is permissive to the spread of virus from the infected to the uninfected eye. In the case in which HSV-1 is injected into the anterior chamber of one eye of a mouse, viral progeny traverse the central nervous system and reach the contralateral optic pathways. Within 7 days of anterior chamber injection, large numbers of viral progeny descend via the optic nerve into the contralateral eye and trigger acute necrosis of the retina. The affected retina becomes heavily infiltrated with polymorphonuclear neutrophils and mononuclear cells. Because this pattern only occurs in immunologically competent mice, the pathogenesis is believed to involve virus-specific immune effector mechanisms. Mice with ARN acquire virus-specific antibodies and CD8+ cytotoxic T cells, and either or both of these effectors could cause the destruction of the virus-infected retina. If this proposed pathogenic mechanism is operative in VZV-ARN, a way to prevent or halt the disease from further progression might be to confer virus-specific DH reactivity on the affected patient. Experiments to test this possibility have not yet been tried in the mouse model system.

Because the incidence of positive skin tests to VZV antigens is so high in the healthy population of Japan, the change observed in VZV DH reactivity in one group

©2001 American Medical Association. All rights reserved.
of patients with VZV-ARN warrants comment. Of subjects who initially had ARN and no VZV-specific DH, almost all displayed VZV DH when tested 3 months later, as their ocular disease resolved. This interesting result must be considered in the context of studies of ACAID in mice. Kosiewicz et al reported that DH to an antigen could be suppressed in sensitized mice if the same antigen is injected into the anterior chamber of the eye. That is, ACAID can be imposed on an already established state of specific immunity. Evidence indicates that most Japanese people possess DH directed at VZV, including individuals destined to develop VZV-ARN. Reasoning from the mouse experiments, we propose that idiopathic reactivation of VZV in the anterior segment of one eye of laboratory rodents but not in humans because the definitive antigenic stimulus to ACAID is removed, and VZV-specific DH can reemerge, as observed in our patients.

Anterior chamber–associated immune deviation has been widely studied in mice, rats, guinea pigs, and rabbits, implying that it is not a phenomenon restricted to laboratory rodents. Eichhorn et al reported in 1993 that ACAID was induced in adult cynomolgus monkeys by intraocular injection of ovalbumin. This finding demonstrates the principle that ACAID can occur in primates. We believe that the results reported herein provide the first evidence compatible with the existence of an ACAID mechanism in humans. Our evidence, like that of Eichhorn et al, is circumstantial. Experimental proof of the existence of ACAID is possible to obtain in laboratory animals but not in humans because the definitive experiments involve the transfer of regulatory T cells from one individual to another.

Accepted for publication February 1, 2001.

We thank Toshihiro Ichikawa, MD, PhD, and Gaku-shi Matsuura, MD, PhD, (Tokyo Medical University) for valuable advice and Hiroshi Minoda, MD, PhD, (Tokyo Medical University) for expert assistance with PCR.

Corresponding author and reprints: Takeshi Kezuka, MD, PhD, Department of Ophthalmology, Tokyo Medical University, 6-7-1, Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan (e-mail: tkezuka@tokyo-med.ac.jp).

REFERENCES