Association Between Anticonvulsant Hypersensitivity Syndrome and Human Herpesvirus 6 Reactivation and Hypogammaglobulinemia

Yoko Kano, MD; Miyuki Inaoka, MD; Tetsuo Shiohara, MD

Background: Anticonvulsant hypersensitivity syndrome (AHS) is a life-threatening, drug-induced, multiorgan system reaction. The identification of predisposing factors is clearly needed to predict the incidence and outcome of AHS; attention has recently been focused on reactivation of human herpesvirus 6 (HHV-6).

Objective: To determine whether immunosuppressive conditions that can allow HHV-6 reactivation could be specifically detected in association with the onset of AHS.

Design: We analyzed patients with AHS who were treated during 1997-2002. Two groups of patients receiving anticonvulsants served as controls.

Setting: Department of Dermatology, Kyorin University School of Medicine, Tokyo, Japan.

Patients: Ten patients with AHS.

Main Outcome Measures: The results of serologic tests for antibody titers for various viruses, including HHV-6, HHV-6 DNA detection by real-time polymerase chain reaction, immunoglobulin levels by turbidimetric immunoassay, IgG subclass levels by nephelometry, and CD19+ B-cell counts by flow cytometric analysis, were sequentially assessed.

Results: Serum IgG levels (mean, 745 mg/dL) and circulating B-cell counts (mean, 88/µL) in patients with AHS were significantly decreased at onset compared with control groups (P<.001 and P=.007, respectively). These alterations returned to normal on full recovery. Reactivation of HHV-6 as judged by a greater than 4-fold increase in HHV-6 IgG titers was exclusively detected in most patients with AHS associated with decreased IgG levels and B-cell counts.

Conclusions: A decrease in immunoglobulin levels and B-cell counts can be associated with HHV-6 reactivation and the subsequent onset of AHS. These immunological alterations might be a useful predictor of the development of AHS.

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ANTICONVULSANT HYPERSENSITIVITY syndrome (AHS) is a life-threatening, drug-induced, multiorgan system reaction characterized by rash, fever, tender lymphadenopathy, hepatitis, and leukocytosis with eosinophilia.¹⁻⁹ The hallmark of the syndrome is the long interval between drug introduction and onset. Unlike other drug reactions, this syndrome typically appears after a prolonged exposure to the drug, which can be 4 weeks or more. It is not related to dosage or serum concentration of the drug.⁶

Because the mortality rate is as high as 38%,³⁸ it is important to predict the onset of this potentially fatal syndrome. Despite progress in the diagnosis, previous studies have failed to determine whether patients who are receiving anticonvulsants are at greater risk of developing this syndrome. Recent studies, including our own, have provided evidence to indicate that reactivation of human herpesvirus 6 (HHV-6), a typical β-herpesvirus implicated in the pathogenesis of certain immunologic disorders such as graft-vs-host disease,²⁶ contributes to the development of AHS.¹¹⁻¹³ However, several questions have arisen, such as why of all similarly treated patients only approximately less than 1% develop AHS after the prolonged induction period,¹⁶ why these structurally diverse drugs create similar symptoms, and whether HHV-6 reactivation is a causal factor or a consequence of AHS.¹⁷⁻¹⁹ We therefore need to elucidate the biological basis for HHV-6 reactivation in AHS. Because the virus seems to be reactivated only under immunosuppressive conditions,²⁰⁻²¹ initial attention was focused on the question of...
whether immunosuppressive conditions that can allow reactivation of HHV-6 could be induced only in patients with AHS and temporarily associated with the onset of the syndrome.

In a review of the reported cases,22-29 we noted that anticonvulsants, allopurinol, and sulfasalazine, all of which have been shown to cause AHS or AHS-like syndrome, can potentially induce transient hypogammaglobulinemia in susceptible patients and animals. Anecdotal case reports30 also revealed that transient hypogammaglobulinemia with absence of circulating B cells can be observed in association with development of AHS-like symptoms. Because declining levels of immunoglobulin with a decrease in B-cell counts have been reported to occur long after starting anticonvulsant therapy, drug-induced hypogammaglobulinemia could provide an explanation for why there is a long interval between drug introduction and the onset of the syndrome. However, because the association between the decreased serum immunoglobulin levels and the onset of AHS has not been well characterized in many patients with AHS and controls, it remains to be determined whether drug-induced hypogammaglobulinemia is a prerequisite for not only onset of AHS but also HHV-6 reactivation.

To investigate whether a decrease in serum immunoglobulin levels was associated with onset of AHS and HHV-6 reactivation, 2 groups of controls were enrolled. One group received anticonvulsants for more than 3 months and did not develop any rashes, and the other received anticonvulsants and developed morbilliform eruptions without any severe systemic manifestations.

### METHODS

**PATIENTS WITH AHS**

Between 1997 and 2002, 10 patients with AHS (6 men, 4 women; mean age, 50.6 years; age range, 26-73 years) were admitted to our university hospital. Criteria for the diagnosis of AHS were high fever (temperature $\geq 38°C$), a widespread erythematous eruption, lymphadenopathy, leukocytosis (white blood cell count $\geq 6000$ µ/L) with atypical lymphocytosis and/or eosinophilia, and liver dysfunction (abnormal alanine aminotransferase levels $\geq 6000$ U/L) with atypical lymphocytosis and/or eosinophilia, and liver dysfunction (abnormal alanine aminotransferase levels, $\geq 3$ times the reference range [3-30 U/L]). Clinical and laboratory findings are summarized in Table 1. The imputable drugs were carbamazepine (6 cases), phenytoin (1 case), phenobarbital (2 cases), and zonisamide (1 case). In these patients, the anticonvulsants were initiated for prevention of epileptic fits. Case 1 had a brain aneurysm. Cases 2, 3, 6, 8, 9, and 10 had psychiatric diseases, such as schizophrenia, delusional condition, and neurosis. Cases 4 and 5 underwent brain surgery for tumor and aneurysm. Case 7 had a history of bacterial brain abscess. The duration between the first drug intake and onset of rashes varied between 19 and 54 days (mean, 35 days); the interval between onset of rashes and
PERIPHERAL BLOOD MONONUCLEAR CELLS

On the day of collection, peripheral blood mononuclear cells (PBMCs) were isolated from the blood, divided into 2 aliquots for flow cytometric analysis and polymerase chain reaction (PCR), and then stored at −80°C. Serum samples were divided into 3 aliquots, which were stored at −80°C for the following analyses.

Serum immunoglobulin levels (IgG, IgA, and IgM) in samples taken at various time points were determined by turbidimetric immunoassay. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete recovery. IgG subclasses were determined by nephelometry in patients with AHS at admission and after complete resolution.

STATISTICAL ANALYSIS

All results were expressed as mean ± SD. The t test (unpaired) was used to assess the analysis of variable values, with P < .05 considered statistically significant. The t test (paired) was used to assess the alterations of serum immunoglobulin levels.

Table 1 and Table 2 give the serum immunoglobulin (IgG, IgA, and IgM) levels in patients with AHS measured on admission and in control patients. A considerable decrease in serum IgG levels (745 ± 163 mg/dL) was noted in patients with AHS compared with those in the 2 control groups (1210 ± 219 mg/dL in control group 1 and 1590 ± 248 mg/dL in control group 2). Serum IgA and IgM levels also showed similar trends toward a decrease in patients with AHS. Because the lowest IgG levels were detected one day and several days after admission in cases 9 (Figure 1) and 10 (data not shown), respectively, serum IgG levels seemed to continue to decrease for at least several days after drug therapy was discontinued. As shown in Table 1 and Figure 2, circulating CD19+ B-cell counts were also profoundly de-

RESULTS

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creased (88±60/µL) in patients with AHS on admission compared with those in control group 1 (247±131/µL). The decreased IgG levels began increasing approximately 14 days after the cessation of the imputable drug and continued to increase, reaching the normal range on full recovery (Figure 3). The IgA and IgM levels also showed similar trends. Because the IgG isotypes produced can be differentially regulated, it was important to determine whether continued anticonvulsant therapy could induce the net decrease in total IgG response by altering the isotype distribution in susceptible individuals. As given in Table 3, all IgG subclass levels were decreased on admission but returned to normal on full recovery. Although IgG1 and IgG3 production was most severely affected at onset of the syndrome, there were no significant differences in their isotype distribution. In 5 patients with AHS (cases 1, 4, 8, 9, and 10) tested, circulating B-cell counts were increasing toward normal 3 weeks after onset, at which time full recovery occurred (data not shown).

Our previous study demonstrated that a marked increase in anti–HHV-6 IgG titer was noted 18 days after onset of the syndrome, and other investigators also reported that serum samples obtained up to 2 weeks after onset had little or no detectable increase in IgG titer. Our results, given in Table 1, indicated that a considerable increase in anti–HHV-6 IgG levels was only detectable in serum samples obtained more than 3 weeks after onset but not in those obtained 2 weeks after onset (cases 3 and 8). The 2 cases revealed negative results on 2 samples, 2 weeks apart, probably due to the inappropriate timing of sampling; serum samples obtained more than 3 weeks after onset were not available in the 2 cases. More than 4-fold IgM titer increases were never found in any serially studied cases. In cases 7, 9, and 10, real-time PCR detected more than 10² copies of HHV-6 DNA in PBMCs obtained approximately 2 weeks after onset but not in samples obtained at the time of admission. Detection of HHV-6 DNA in PBMCs apparently preceded the dramatic increase in anti–HHV-6 IgG titers. Findings for HHV-6 DNA were negative by PCR in sequential blood samples obtained from controls. Because the decrease in serum IgG levels and B cells had been detectable at the time of the first visit, these alterations clearly preceded increases in HHV-6 IgG titers and detection of HHV-6 DNA. Treatment with systemic corticosteroids did not seem to influence the HHV-6 IgG titers and the rate of HHV-6 DNA detection. No significant increase in anticytomegalovirus and anti–Epstein-Barr virus IgM and IgG levels was detected during the same period in all cases except cases 1 and 9; their cytomegalovirus IgM serologic titers continuously increased throughout the observation period. Thus, there was a strong relation of the decrease in serum IgG levels and B cells to reactivation of HHV-6 and onset of AHS.

**COMMENT**

Because the contribution of HHV-6 reactivation in the pathogenesis of AHS is only now being appreciated, its significance in the pathogenesis of AHS and its interaction with AHS are still a matter of debate. Although our study argued strongly that HHV-6 reactivation is critical to the development of AHS, others have cast doubt on this no-
The relationship between HHV-6 and AHS is highly complex, with evidence that HHV-6 reactivation can represent either a cause or a mere consequence of immune dysfunction occurring during AHS. This study clearly shows that HHV-6 reactivation as judged by a more than 4-fold increase in HHV-6 IgG titers and HHV-6 DNA positivity was exclusively detected in patients with AHS associated with decreased serum IgG levels and decreased B-cell counts but not in those receiving the same drug without these alterations. More important, neither decreases in serum IgG levels and B-cell counts nor HHV-6 reactivation was observed in other severe adverse drug reactions, such as Stevens-Johnson syndrome (n=3; mean of serum IgG level, 1522±370 mg/dL) and toxic epidermal necrolysis (n=1; serum IgG level, 1050 mg/dL) (Y.K. and T.S., unpublished data, 2001). These results indicate that selective reactivation of HHV-6 associated with decreased serum IgG levels and decreased B-cell counts might be specific to AHS. Because no gold standard for the diagnosis of AHS exists, this would be a reliable marker for an increased risk of developing AHS in patients receiving anticonvulsants.

Nevertheless, one may argue that a significant increase in anti--HHV-6 IgG titers and PBMC HHV-6 DNA levels has not been constantly detected in patients with AHS. The realization that the increase in HHV-6 antibody titers and DNA levels was only detectable more than 3 weeks after onset of the symptoms in most cases with AHS provides a plausible explanation for why so many negative findings from serum and PBMC samples were found in patients with AHS during the first few weeks after onset. The apparent certain timing of the increase in HHV-6 antibody titers, that is, 3 to 4 weeks after onset, regardless of the clinical course and treatment, could now be interpreted as an indication that HHV-6 reactivation is not a mere consequence of but a prerequisite for the development of AHS and that a massive expansion of HHV-6-specific and nonspecific bystander CD8+ and CD4+ T cells in response to HHV-6 reactivation could be responsible for a stepwise development of multiple organ failure in AHS. This view is supported by a recent report by Desachy et al.33 They demonstrated that the incidence of HHV-6 reactivation was markedly greater in patients with multiple organ failure syndrome simulating AHS than in control patients. The question then arises why reactivation of HHV-6 can be only detectable more than 3 weeks after onset of symptoms. In this regard, there is some evidence suggesting that HHV-6 reactivation might initially occur in limited sites in the body other than the blood, such as liver and lymph nodes; however, as far as we are aware, no attempt was made to detect HHV-6 DNA in these sites. Alternatively, it is also possible that HHV-6 DNA could be transiently detectable before onset of clinical symptoms if it were possible to obtain samples from blood or these sites.

For the first time to our knowledge, our data provide a satisfying explanation for why continuous anticonvulsant therapy can cause a severe multiorgan system reaction that resembles severe infectious mononucleosis in susceptible individuals. HHV-6 reactivation associated with the development of AHS could occur as a result of transient immune dysfunction, such as decreased IgG production probably induced by continuous anticonvulsant therapy. It is generally believed that T cells prevent reactivation of latently infected cells, whereas antibodies prevent the dissemination of reactivating lytic virus. However, the mechanisms crucial for viral control may differ, depending on the cell types infected and the virus. Recent studies30 have demonstrated the possible role of B cells in viral clearance during a primary infection. Surprisingly, B cells are required for the clearance of infectious virus from the central nervous system but not the liver, suggesting that the requirement for B cells in clearance of a viral infection can be organ specific.32,33 Thus, these studies showing the importance of antibodies and B cells in controlling the persistent stage of viral infection are consistent with our data suggesting a contribution of serum IgG and B cells to prevent HHV-6 reactivation. However, these findings could alternatively be interpreted as indicating that decreased IgG production merely represents an early manifestation of AHS but not an etiologic factor. Although this possibility cannot be definitively excluded, this is less likely because hypogammaglobulinemia in patients receiving anticonvulsants was not always followed by the development of AHS. In this regard, we assume that, in addition to HHV-6 reactivation, activation of drug-specific T cells plays an indispensable part in a multiple process, resulting in the development of AHS. This ordered sequence of events is thought to be orchestrated by interactions among many immune cells and viruses. Thus, decreased IgG production would be essential but not sufficient for the development of AHS. Hypogammaglobulinemia is also unlikely to be a simple consequence of facial or generalized edema often associated with AHS, because hypogammaglobulinemia was detected in patients without edema (Table 1).

We were initially surprised to note that anticonvulsants, allopurinol, and sulfasalazine, all of which have been shown to cause AHS or AHS-like syndrome, can potentially transiently induce hypogammaglobulinemia in susceptible patients and animals. Hypogammaglobulinemia is an early manifestation of AHS but not an etiologic factor. A hypogammaglobulinemia can be attributed either to a direct consequence of transient hypogammaglobulinemia, no satisfying explanation has yet been offered for why decreased humoral reactions could result in the development of AHS. Our analysis provides evidence to indicate that long-term use of anticonvulsants, when acting through a direct immunosuppressive effect on B cells and subsequent decreased immunoglobulin production, may induce selective reactivation of HHV-6 in susceptible individuals. In support of this notion, transient hypogammaglobulinemia followed by HHV-6 reactivation was also detected on admission in 3 patients with allopurinol hypersensitivity syndrome (mean serum IgG level, 728±134 mg/dL) analogous to AHS but not in control patients who were taking allopurinol for more than 3 months without any adverse reactions (mean serum IgG level, 1263±187 mg/dL) (Y.K. and T.S., unpublished data, 2001), a finding identical to that of patients with AHS. In addition, a decreased serum IgG level was also observed in a patient with rheumatoid arthritis and Sjogren syndrome who developed hypersensitivity syndrome analogous to AHS after treatment with sulfasalazine for 27 days. These immuno-
logical findings suggest that decreased B-cell counts and immunoglobulin production and subsequent HHV-6 reactivation could be involved in onset of AHS. AHS could result when long-term use of anticonvulsants causes a transient decrease in B-cell counts and immunoglobulin production, via reactivation of HHV-6, in addition to the presence of drug-specific T cells. The time required to develop AHS after starting anticonvulsant therapy could reflect the time needed for immunoglobulin levels to decrease below a threshold level.

Whatever the mechanisms involved, confirmation and extension of our data point to the therapeutic use of intravenous immunoglobulin (IVIG) containing high HHV-6 IgG titers for the treatment of severe AHS. Indeed, cases 5 and 10 have been treated with IVIG containing high HHV-6 IgG titers, and the results have been encouraging. Administration of IVIG has recently been shown to result in rapid improvement in a pediatric patient with AHS, although it was not mentioned whether the IVIG contained high HHV-6 IgG titers. In view of our findings, the therapeutic effect of IVIG in AHS could be attributed at least in part to functional capabilities to prevent HHV-6 reactivation of anti–HHV-6 antibodies contained in pharmacologically prepared human IgG. These considerations are compatible with a recent observation that in vivo protection against virus was independent of immunoglobulin subclass, avidity, neutralization rate constant, and in vitro neutralization activity but dependent simply on a minimum serum concentration.

In conclusion, measurement of serum immunoglobulin levels and B-cell counts would be useful for the early detection of AHS in patients receiving anticonvulsant therapy, although long-term studies with larger numbers of patients will be necessary to confirm our findings. If anticonvulsant therapy is complicated by these immunological alterations, a prompt decision is necessary regarding drug withdrawal to avoid a potentially fatal outcome.

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Corresponding author and reprints: Yoko Kano, MD, Department of Dermatology, Kyorin University School of Medicine, 6-20-2 Shinkawa Mitaka, Tokyo, 181-8611, Japan.

REFERENCES


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