Sin Nombre Virus–Specific Immunoglobulin M and G Kinetics in Hantavirus Pulmonary Syndrome and the Role Played by Serologic Responses in Predicting Disease Outcome

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Background. Sin Nombre virus (SNV) is the primary cause of hantavirus pulmonary syndrome (HPS) in the United States. Although other studies have demonstrated a possible association between neutralizing antibody titers and the severity of HPS, the exact nature of serologic responses and their association with outcomes have not been fully characterized.

Methods. We examined immunoglobulin M (IgM) and immunoglobulin G (IgG) serologic responses in 94 clinical samples from 81 patients with confirmed HPS. We further compared a subset of 31 patients with fatal HPS and 20 surviving patients for whom samples were available within a week after the onset of HPS.

Results. SNV-specific IgM antibodies displayed a trend suggesting an early peak, whereas IgG antibody values peaked later. Among individuals with samples from the first week after the onset of HPS, all surviving patients had SNV-specific IgG responses, compared with <50% of patients with fatal HPS, and the distribution of IgG responses was significantly higher in surviving patients.

Conclusions. Production of SNV-specific IgM antibodies occurs early during the clinical course of HPS, whereas production of IgG antibodies may be more protracted. The presence and overall distribution of higher IgG antibody titers in surviving patients with HPS suggests that production of SNV-specific IgG may be a strong predictor of favorable outcomes.

Viruses of the genus Hantavirus are the etiologic agents of 2 human diseases, hantavirus pulmonary syndrome (HPS) (also referred to as hantavirus cardiopulmonary syndrome) and hemorrhagic fever with renal syndrome (HFRS). Owing to the distribution of their rodent reservoirs, HPS is a new-world disease, occurring in North, Central, and South America, and is associated with a number of hantavirus species [1–3]. In contrast, HFRS cases occur across a large portion Europe and Asia, with only a few cases of Seoul virus infection in the Americas. Although varying in their pathogenic presentation in humans, viral species responsible for HFRS include Hantaan, Dobrava, Puumala, and Seoul viruses [2, 4].

HPS is a severe disease that affects the respiratory and circulatory systems. After a short viral prodrome, the hallmark of HPS is the rapid onset of pulmonary edema, often resembling acute respiratory distress syndrome [5, 6]. In the United States, the case fatality rate for HPS is >35% [6] (authors’ unpublished data). Most cases of HPS in the United States are due to Sin Nombre virus (SNV) infection [7] and occur in the western portion of the country [3, 6] (authors’ unpublished data), corresponding to the distribution of the reservoir host, the deer mouse (Peromyscus maniculatus) [3, 8].

The primary impact of new-world hantaviruses during HPS occurs in the lungs. The characteristic pulmonary edema is believed to be due to increased vas-
cular permeability and may result from viral infection of endothelial cells [9, 10], although animal studies involving Cholco virus have demonstrated infection of pulmonary endothelial cells with the absence of edema [11]. Production of cytokines may also contribute to pulmonary edema [12, 13]. During HPS, virus-specific immunoglobulin M (IgM) and immunoglobulin A (IgA) responses tend to develop rapidly, followed by an increase in immunoglobulin G (IgG) antibody titers [14]. Among survivors, neutralizing antibody titers persist for long periods of time [15]. In addition, other studies have suggested that there is a relationship between low neutralizing antibody titers and increased severity of HPS [16]. To further characterize the kinetics of the human serologic response to hantavirus during HPS and the relationship between serologic response and severity of disease, we examined data from 81 patients with confirmed HPS for whom samples were submitted to our laboratory for diagnostic testing.

METHODS

Data collected for this study were obtained through routine public health surveillance. Samples submitted to the Centers for Disease Control and Prevention for HPS testing were assayed by IgM capture and IgG enzyme-linked immunosorbent assays (ELISAs), as described elsewhere [17]. The viral antigens for the IgM and IgG ELISAs were, respectively, a whole-virus preparation of SNV from cell culture and an Escherichia coli-expressed complete nucleoprotein (control mock virus antigen was prepared in the same manner). The seroreactivity measurements were based on polyclonal antibody response to the virus. Although these IgM and IgG ELISAs are cross-reactive against all new-world hantaviruses, this study was limited to data for patients whose HPS was believed to be due to SNV.

Serum samples were tested against SNV antigen and control antigen using 4-fold dilutions of serum, from 1:100 to 1:6400. Adjusted optical density (OD) values for individual serum dilutions were calculated by subtracting the SNV antigen–specific OD value from the control antigen OD value. Individual dilutions with an adjusted OD of >0.20 for the IgG assay and >0.10 for the IgM assay were considered positive, and the adjusted sum OD value was calculated by adding the adjusted OD values for all dilutions of a given serum sample. Positive serum samples were defined as those with a titer of ≥400 (reported as reciprocal values) and an adjusted sum OD value of ≥0.91 for the IgG assay or ≥0.45 for the IgM assay. Epidemiologic and clinical data for HPS cases in this study were collected on case investigation forms and maintained in our HPS registry.

RESULTS

We evaluated the serologic responses in a group of samples from patients with confirmed HPS. Samples for this study were limited to those from patients whose HPS was probably due to SNV infection, based on the geographic location of the potential infection (and rodent exposure, if known). In total, we evaluated serologic responses among 94 samples acquired from 81 patients with HPS. The median age of patients was 36 years (range, 8–70 years), and 56 patients were male. HPS was fatal in 34 patients, and 47 patients survived. Samples were primarily collected during the acute phase of infection, with the majority acquired within a week after the onset of symptoms; 9 samples were collected >3 weeks after the onset of disease (range, 23–51 days after onset).

Regardless of the time of collection relative to the onset of symptoms, all samples displayed a positive SNV-specific IgM titer. Even within the first few days after the onset of symptoms, the majority of samples had IgM titers ≥6400 (Figure 1A). IgM titers, as well as adjusted sum OD values for IgM, displayed a slight upward trend early during infection, with adjusted sum OD values peaking within 11–14 days after the onset of symptoms (Figure 1B). Overall trends in IgM titer and adjusted sum OD values did display a slight downward trend for samples collected at longer intervals after onset; 2 samples (collected 46 and 49 days after onset) had IgM titers of only 400.

In contrast to IgM titers, a sizable portion of early samples did not have an SNV-specific IgG titer (Figure 1C). Compared with values for IgM, median IgG titers, as well as adjusted sum OD values for IgG (Figure 1D), displayed a trend toward increasing values for a longer interval after the onset of disease.

To compare serologic responses between individuals with fatal and those with nonfatal outcomes, we examined serologic values for patients with samples collected within 1 week after the onset of symptoms. For 3 patients from whom multiple samples were collected within the first week after onset, we included only the first sample collected. Thirty-one patients with fatal HPS and 20 surviving patients had samples collected within 1 week after the onset of symptoms; no substantial difference was noted in the interval from onset to sample collection between these groups (mean for patients with fatal HPS vs survivors, 4.0 vs 4.5 days).

All patients with HPS evaluated had detectable SNV-specific IgM antibodies. However, virtually all surviving patients (18/20) had SNV-specific IgM titers ≥6400, whereas 10 of 31 with fatal HPS had IgM titers <6400 (Figure 2A), and the distribution of SNV-specific IgM titers was significantly higher in the surviving group (P = .041, Wilcoxon rank sum test). Similarly, the distribution of adjusted sum OD values for IgM tended to be higher in the surviving group (Figure 2B); however, this difference was marginal (P = .107, Wilcoxon rank sum test).

All surviving patients had an SNV-specific IgG titer (median titer, ≥6400). In contrast, more than half of the patients with fatal HPS (16/31) did not have an SNV-specific IgG titer, and
Figure 1. Sin Nombre virus (SNV)–specific immunoglobulin M (IgM) titers (A), adjusted sum optical density (OD) values for IgM (B), SNV-specific immunoglobulin G (IgG) titers (C), and adjusted sum OD values for IgG (D), by time of collection relative to the onset of symptoms. Black lines denote median values.

The distributions of SNV-specific IgG titers differed significantly between these groups (P < .001, Wilcoxon rank sum test) (Figure 2C). In addition, this difference remained when we compared adjusted sum OD values for IgG between these 2 groups (P < .001, Wilcoxon rank sum test) (Figure 2D).

To further evaluate the potential relationship between serologic responses and the severity of disease among surviving patients, we compared serologic parameters between surviving patients who required intubation (n = 16) and those who did not (n = 25). No significant differences between these groups were observed in SNV-specific IgM titer, adjusted sum OD values for IgM, IgG titer, or adjusted sum OD values for IgG (data not shown).

**DISCUSSION**

In this study, we evaluated serologic responses to SNV in a large group of patients with HPS. We observed an early development of and peak in SNV-specific IgM antibodies in these patients. Although the development of IgG responses was more variable relative to time, the overall trend was suggestive of an IgG response that peaks during the first few weeks after infection and remains high thereafter. These trends are consistent with findings reported by others for both hantavirus infections resulting in HPS and those resulting in HFRS [14, 18–22]. Although we do not have any long-term follow-up data in this study, others have reported the persistence of high titers of hantavirus-specific antibodies for long periods of time after recovery from HPS or HFRS [15, 19–21].

The variability in IgG titers among the early samples (with some having high IgG titers even during the first few days of symptoms) may be partially explained by the variable incubation period of HPS, reported to be in the range of ∼1–5 weeks [23]. For instance, an individual who experiences a long incubation period may have ample time to develop an SNV-specific IgG response before the onset of symptoms.

When SNV-specific IgG responses occurring within the first week after onset were compared between surviving patients and patients with fatal HPS, we noted significantly higher titers and adjusted sum OD values among the survivors. This finding implies that the ability to mount a strong IgG response during the early stages of HPS is a strong predictor of survival. In addition, although differences were not as substantial, our data
suggest that stronger IgM responses may also be associated with favorable HPS outcomes. Bharadwaj et al [16] have reported an association between severe HPS and lower neutralizing antibody titers. In contrast, among surviving patients in our study we did not observe any difference in IgM or IgG responses between mild and severe cases (categorized on the basis of whether intubation was required). The discordance between the findings of Bharadwaj et al and our data may be partially explained by the fact that the former study combined severe HPS cases into a single group (regardless of survival status) for comparison with mild cases. On the basis of data from the present study, neutralizing antibody titers would clearly be expected to be higher in survivors than in patients with fatal HPS.

HPS has predominantly respiratory manifestations, and, consistent with the site of pathology, IgA responses during HPS have been described [14, 16, 18]. Although the exact role played by IgG antibody responses in the control of SNV infection is not completely clear, a couple of possible explanations may account for our finding that the IgG response is a strong predictor of HPS survival. First, high levels of viremia have been reported in patients with HPS [24], and IgG antibodies would thus be expected to play a role in the clearance of disseminated virus. Second, in addition to IgG, our data also suggested a possible association between IgM responses and survival. Combined, these data may underscore the importance of an overall robust serologic response in the control of HPS. Finally, although we assessed serologic responses relative to time after the onset of symptoms, we do not have data pertaining to time from initial virus exposure. It is possible that samples with higher IgG titers at the onset of symptoms represent HPS cases with a longer incubation period.

Among patients with fatal HPS, death often occurs shortly after hospitalization [6]. Given that no antiviral therapies currently exist, the utility of IgG serologic responses may be limited from a clinical judgment standpoint. However, extracorporeal membrane oxygenation may be efficacious as supportive therapy for patients with severe HPS [25], so serologic values may help inform some clinical decisions.
In conclusion, we assessed the serologic response to SNV in a large number of patients with HPS. Our data demonstrate a relationship between antibody responses and favorable disease outcome and imply that antibody response is a strong correlate of control of hantaviruses during HPS.

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References