

## Serum Levels of Alpha and Gamma Interferons in Hemorrhagic Fever with Renal Syndrome

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### ABSTRACT

**Hemorrhagic fever with renal syndrome is an acute viral disease caused by *Hantavirus*. On the basis of clinical observation, the illness is divided into five sequential stages: febrile, hypotensive, oliguric, diuretic, and convalescent. Because interferons can be induced by viruses, and because their stimulating effects on immune cells can alter the course of viral infections, we examined the presence of alpha interferon (IFN- $\alpha$ ) and gamma interferon (IFN- $\gamma$ ) in 276 serum samples collected from 110 patients during the Korean Conflict. We tested these sera for IFN- $\alpha$  by bioassay with bovine kidney MDBK cells, and for IFN- $\gamma$  by a sandwich ELISA with antibodies specific for human IFN- $\gamma$ . We found variable, but persistently elevated levels of IFN- $\gamma$  throughout the various phases of the disease, which suggested persistent immune activation through convalescence. Moderate levels of IFN- $\alpha$  were found in all stages of infection.**

### INTRODUCTION

**K**orean hemorrhagic fever (KHF) is a member of a group of similar hemorrhagic fevers with renal syndrome that occur throughout northern Asia, Russia, Scandinavia, and western Europe. This disease was initially recognized in Korea during the Korean Conflict, when >3000 cases occurred in United Nations troops. The etiologic agent of KHF remained elusive until 1976, when Hantaan virus was isolated from the Asian field mouse *Apodemus agrarius coreae*, and shown to be casually related to this disease (7). Subsequently, laboratory diagnostic tests were developed to measure specific anti-hantaviral antibodies in serum (10). Other Hantavirus, Seoul and Prospect Hill viruses, have been isolated from rodents in North America (5,8). There is serologic evidence of asymptomatic infection with Prospect Hill virus among North American mammologists (13), while recent evidence suggests that past Seoul virus infections may be associated with subsequent development of chronic renal disease (1). Since interferons are present during acute viral infections (9), and they are potent immunomodulatory agents, we examined the role of interferons in the antiviral response to Hantaan infection to determine whether they are associated with disease progression. We used specific and sensitive assays to determine if IFN- $\alpha$  and IFN- $\gamma$  were present in a large collection of acute and convalescent sera from serologically confirmed KHF patients.

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## MATERIALS AND METHODS

**Serum collection.** Sera were obtained from June 1952 to July 1953 from 276 patients with a clinical diagnosis of hemorrhagic fever. These samples were collected by the Hemorrhagic Fever Commission and labeled with each patient's name and number, date of collection, volume, and a "DD" number, which represents the "day of disease." The information on the label is the only patient information that we have been able to locate, and we used these dates to calculate days after onset of disease when analyzing data. The sera had been lyophilized in glass ampules, and packed in cardboard boxes in three metal trunks and kept at  $-30^{\circ}\text{C}$ . Sera were rehydrated with sterile, distilled water according to the volume indicated on each label, mixed thoroughly, then transferred to plastic tubes and stored at  $-70^{\circ}\text{C}$  until tested. Different aliquots of these sera were previously examined and shown by enzyme immunoassays to contain specific IgM and IgG anti-Hantaan virus antibodies. At least one serum sample from each patient was shown to contain neutralizing antibodies specific for Hantaan virus (6).

**IFN- $\alpha$  determination.** Serum levels of IFN- $\alpha$  were determined by measuring the reduction of the cytopathic effect of vesicular stomatitis virus (VSV, New Jersey strain) on bovine kidney cells (MDBK). Duplicate, serial twofold dilutions of human serum samples were made in 96-well microtiter plates. Approximately  $5 \times 10^4$  cells were added to each well. Plates were incubated at  $37^{\circ}\text{C}$  in 5%  $\text{CO}_2$  for 24 hr and then challenged with  $7 \times 10^5$  PFU of VSV. After 48 hr incubation, cell controls showed 100% cytopathic effect. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (0.5 mg/ml) was then added to each well to stain live cells and the plates were incubated for an additional 3 hr. Cell supernatants were decanted and the dye was extracted by incubation with 200  $\mu\text{l}$  of acidified isopropanol. The OD at 570 nm of each well was determined by a ELISA reader (Dynatech, Alexandria, VA). The number of units of IFN per ml for each sample was estimated by comparing sample titers with the reference standard IFN- $\alpha$  (Collaborative Research, Boston, MA). The lowest concentration of IFN- $\alpha$  detectable was 2 U/ml in this assay and a control group of four normal adult donors had no detectable serum IFN- $\alpha$ . The specificity of this bioassay for IFN- $\alpha$  was verified by the neutralization of the antiviral effect of an IFN- $\alpha$  standard by an antiserum against human IFN- $\alpha$  (Boehringer-Mannheim, Indianapolis, IN). In addition, IFN- $\gamma$  at 1000 U/ml lacks antiviral activity in this assay.

**IFN- $\gamma$  determination.** Serum IFN- $\gamma$  was measured by ELISA as follows: 96-well plates were coated with murine monoclonal anti-IFN- $\gamma$  (Boehringer Mannheim, IN). After blocking nonspecific binding with 1% bovine serum albumin, 25 to 100  $\mu\text{l}$  of sample was added. IFN- $\gamma$  was then detected by the sequential addition of polyclonal rabbit anti-IFN- $\gamma$  (Interferon Science, New Brunswick, NJ) and peroxidase conjugated anti-rabbit antibody (Boehringer Mannheim, IN). Bound enzyme was measured at 450 nm (MR600 Microplate Reader, Dynatech, Alexandria, VA) by using 3,3',5,5'-tetramethylbenzidine and hydrogen peroxide as substrates (Kirkegaard and Perry Lab, Gaithersburg, MD). A standard curve of recombinant human IFN- $\gamma$  (Collaborative Research, MA) was prepared for each ELISA. The sensitivity of the ELISA was 1 U/ml. Serum from two normal adult blood donors had a mean value of  $3 \pm 1$  U/ml (mean  $\pm$  SD).

In the evaluation of samples for elevated levels of IFN- $\alpha$  and IFN- $\gamma$ , 6 and 14 U/ml were used as baselines because, based on our *in vitro* studies, these interferons at these doses or below show no antiviral activity in human peripheral blood mononuclear cells (unpublished data).

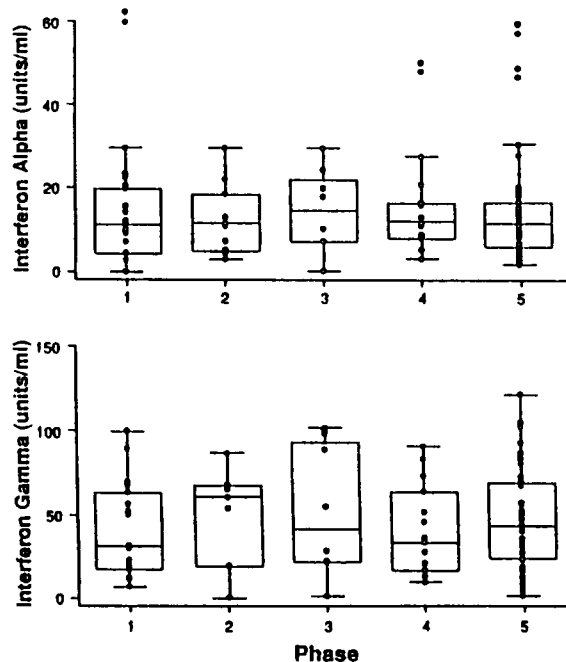
**Statistical analysis.** All statistical analyses were carried out by using the State software package (Computing Resource Center, Santa Monica, CA).

## RESULTS

A total of 276 serum samples from 109 patients was studied and 77 and 75% of samples had levels of IFN- $\alpha$  ( $>6$  U/ml) and IFN- $\gamma$  ( $>14$  U/ml) which, in *in vitro* studies, commonly possess antiviral activity in peripheral blood mononuclear cells. Because we were interested in following the levels of these IFNs with the progression of the disease through its defined clinical stages, 98 samples from 32 patients with multiple ( $\geq 3$ ) time points were chosen for detailed statistical analysis. However, findings obtained for this subset were generally similar to those in the entire set.

The day of disease of KHF was divided into five phases according to the known clinical stages of epidemic

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**FIG. 1.** Serum IFN- $\alpha$  and IFN- $\gamma$  from KHF patients over five clinical phases. Normal IFN- $\alpha$  = 0 U/ml. Normal IFN- $\gamma$  = 3 U/ml. Phase 1, febrile phase, 0–5 days. Phase 2, hypotensive phase, 6–9 days. Phase 3, oliguric phase, 10–13 days. Phase 4, diuretic phase, 14–21 days. Phase 5, convalescent phase, 22–54 days.

hemorrhagic fever (11,12). They are defined as follows: phase 1 (febrile, 0 to 5 days), phase 2 (hypotensive, 6 to 9 days), phase 3 (oliguric, 10 to 15 days), phase 4 (diuretic, 16 to 21 days), and phase 5 (convalescent, 22 to 54 days).

Figure 1 represents serum levels of IFN- $\alpha$  at these various phases. Within this group of 98 samples, 75% of samples showed elevated levels. Median levels (from 10 to 14 U/ml) were the same throughout the five different stages.

Serum levels of IFN- $\gamma$  in the different phases of disease are shown in Fig. 1 (lower panel). Of the samples 79% had elevated levels >14 U/ml. Median levels were 34, 65, 44, 35, and 45 U/ml for phases 1, 2, 3, 4, and 5, respectively. Although there was no statistically significant difference in the levels of IFN- $\gamma$  in the different phases, a peak level of IFN- $\gamma$  during the hypotensive phase was suggested.

The percentages of samples with elevated levels of IFN- $\alpha$  and IFN- $\gamma$  are shown in Table 1. Elevated levels of both IFN- $\alpha$  and IFN- $\gamma$  occurred in a larger proportion (87%) of samples in the oliguric phase of KHF. There appears to be a downward trend in the proportion of samples with elevated IFN- $\alpha$  in the later stages (diuretic and convalescent phase). The two interferons did not always appear simultaneously.

TABLE 1. PERCENTAGES OF SAMPLES WITH ELEVATED LEVELS OF IFN- $\alpha$  AND IFN- $\gamma$  OVER THE CLINICAL COURSE OF KHF

	<i>Phase</i>				
	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>
Number of samples	22	10	8	15	43
% with elevated IFN- $\alpha$	68	70	87	80	74
% with elevated IFN- $\gamma$	77	70	87	80	81

## DISCUSSION

This study demonstrates that about 75% of sera from KHF patients had elevated levels of serum IFN- $\alpha$  and IFN- $\gamma$ . Recently, high levels of IFN- $\alpha$  and IFN- $\gamma$  were also found in dengue hemorrhagic fever and measles, respectively (4,2). Interferons represent a family of proteins with antiviral as well as immunoregulatory functions. Analysis of the production of IFN- $\gamma$  during viral infections is of great importance because of its role in macrophage activation. Elevated levels of IFN- $\gamma$  may enhance the responsiveness of lymphoid cells to other cytokines and promote inflammatory responses. Recently, IFN- $\gamma$  was shown to upregulate the expression of intercellular adhesion molecule-1 on human renal tubular epithelial cells (3), which likely would facilitate infiltrating T cell attachment and the prolonged presence of IFN- $\gamma$  may therefore lead to immunopathology. We have shown that both interferons were elevated throughout the course of KHF. The persistent presence of IFN- $\gamma$  is particularly interesting as it indicates immune activation that persisted through the convalescent phase.

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## REFERENCES

1. Glass G.E., A.J. Watson, J.W. LeDuc, G.D. Kelen, T.C. Quinn, and J.E. Childs. 1993. Infection with a ratborne hantavirus in US residents is consistently associated with hypertensive renal disease. *J. Infect. Dis.* 167:614-620.
2. Griffin D.E., B.J. Ward, E. Jauregui, R.T. Johnson, and A. Vaisburg. 1990. Immune activation during measles: Interferons and neopterin in plasma and cerebrospinal fluid in complicated and uncomplicated diseases. *J. Infect. Dis.* 161:449-453.
3. Ishikura, H., C. Takahashi, K. Kanagawa, H. Hirata, K. Imai, and T. Yoshiki. 1991. Cytokine regulation of ICAM-1 expression on human renal tubular epithelial cells in vitro. *Transplantation* 51:1272-1275.
4. Kurane, I., B.L. Innis, S. Nimmannitya, A. Nisalak, A. Meager, and P.A. Ennis. 1993. High levels of interferon alpha in the sera of children with dengue virus infection. *Am J. Trop. Med. Hyg.* 48:222-229.
5. LeDuc, J.W., G.A. Smith, and K.M. Johnson. 1984. Hantaan-like viruses from domestic rats captured in the United States. *Am. J. Trop. Med. Hyg.* 33:992-998.
6. LeDuc, J.W., T.G. Ksiazek, C.A. Rossi, and J.M. Dalrymple. 1990. A retrospective analysis of sera collected by the Hemorrhagic Fever Commission during the Korean Conflict. *J. Infect. Dis.* 162:1182-1184.
7. Lee, H.W., P.W. Lee, and K.M. Johnson. 1978. Isolation of the etiologic agent of Korean hemorrhagic fever. *J. Infect. Dis.* 137:298-308.
8. Lee, P.W., H.L. Amyx, R. Yanagihara, D.C. Gajdusek, D. Goldgaber, and C.J. Gibbs, Jr. 1985. Partial characterization of Prospect Hill virus isolated from meadow voles in the United States. *J. Infect. Dis.* 152:826-829.
9. Levin, S., and T. Hahn. 1981. Evaluation of the human interferon system in viral disease. *Clin. Exp. Immunol.* 46:475-483.
10. Meegan, J., and J.W. LeDuc. 1989. Enzyme immunoassays, pp. 83-87. In H.W. Lee and J. Dalrymple (eds.), *Manual of hemorrhagic fever with renal syndrome*. Seoul: WHO Collaborating Center for Virus Reference and Research (HFRS).
11. Sheedy, J.A., H.F. Froeb, H.A. Batson, C.C. Conley, J.P. Murphy, R.B. Hunter, D.W. Cugell, R.B. Giles, S.C. Bershadsky, J.W. Vester, and R.H. Yoe. 1954. The clinical course of epidemic hemorrhagic fever. *Am. J. Med.* 16:619-628.
12. Tsai, T.F. 1987. Hemorrhagic fever with renal syndrome: Clinical aspects. *Lab. Anim. Sci.* 37:419-427.

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13. Yanagihara, R., D.C. Gajdusek, C.J. Gibbs Jr., and R. Traub. 1984. Prospect Hill virus: Serologic evidence for infection in mammalogists. *N. Engl. J Med.* 310:1325-1326.

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