Detection of Antibodies in Serum and Lymphocytes of Patients with Hemorraghic Fever with Renal Syndrome

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Abstract

Hemorrhagic Fever with Renal Syndrome (HFRS) is zoonotic disease, endemic in the republic of Tatarstan. Increased endothelial barrier permeability, resulting in tissue edema, leukocyte migration and hemorrhages, is characteristic pathological finding in HFRS. Cytokine storm hypothesis was suggested to explain hantavirus pathogenesis, where over activation of pro-inflammatory cytokines was suggested to cause endothelial cell damage and increased leukocyte infiltration of target tissue. Hantavirus nucleocapsid (N) protein and glycoprotein proteins (G1 and G2) were suggested to be the most immunogenic viral proteins. However, information on immunogenic epitopes triggering humoral and cellular immune response is limited.

Materials and methods. Blood and serum samples were collected from HFRS patients admitted to Republican Clinical Hospital for Infectious Disease named after Agafonov, Republic of Tatarstan along with controls matched for the region. The Institutional Review Board of the Kazan Federal University approved this study and informed consent was obtained from each study subject according to the guidelines approved under this protocol (article 20, Federal Law "Protection of Health Rights of Citizens of Russian Federation" N323- FZ, 11.21.2011).

Peptide library consisting of overlapping peptides (15 aa; 5 aa overlap) of N, G1 and G2 of Puumala virus (PPUV) were generated. Total of 150 peptides were tested. PUUV was selected because it is endemic in Republic of Tatarstan causing HFRS.

Immunogenic epitopes inducing antibody response in HFRS were analyzed using direct ELISA. Peptides were used to coat the plate (1 µg/well), followed by incubation with HFRS serum. Control serum samples were used to determine peptide non-specific reactivity. Statistical analysis was conducted using Minitab software; differences between medians of HFRS and control groups were analyzed using the Mann-Whitney test for non-parametric data. Data was considered significant at $p \leq 0.05$.

T-lymphocyte epitopes were analyzed using ELISpot assay. Blood from HFRS cases was collected in vacuum tubes containing sodium citrate (Vacuutest, Apexmed). Confirmation of PUUV infection was done by PCR (n=10). Peripheral blood mononuclear cells (PBMC) were isolated using ficoll density gradient separation (p=1,077 g/l). Isolated mononuclear leukocytes were cultured in 96-well plates coated with anti-IFNγ antibody. Leukocytes were incubated with each peptide for 48 hours. PBMC treated with phytohaemoagglutinine (PHA) served as a positive control. Spots were visualized using AEC substrate and counted by direct microscopy.

Results. PUUV RNA was detected in all blood samples. Serum samples from 10 HFRS cases and 10 controls were analyzed on reactivity to the overlapping peptides coding nucleocapsid and G1 and G2 glycoproteins. Reactivity of eleven peptides (150 total) was statistically significant in HFRS serum as compared to controls (Figures 1a, 1b). Interestingly, the intensity of reactivity between HFRS serum and glycoprotein peptides was 1.5±0.2 times higher than that of N protein.

Several G and N protein peptides were identified as inducing IFN-γ production by leukocytes. These peptides are M82 (YTRKACIQLGTEQTC), M69 (TDLELDFSLPSSASY), N3 (ARQKLKDAERAVEVY), N2 (ITRHEQQLVVARQKL), N1 (MSDLTDIQEEITRHE) and M104 (NLVRGQNTVHIVGKG) (Figure 1c).

Collected data revealed that the most immunogenic peptides were M104 (NLVRGQNTVHIVGKG) and N3 (ARQKLKDAERAVEVY) from hantavirus G and N proteins respectively. These peptides revealed strong reactivity in ELISA and ELISpot assays suggesting that they play role in activation of humoral and immune responses.

Conclusion. Data confirms that both N and G proteins of PUUV are immunogenic. New epitopes were identified on N and G proteins which can stimulate humoral and cellular immunity.

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