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Letter to the Editor

Immunopathogenesis of hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome: Do CD8$^+$ T cells trigger capillary leakage in viral hemorrhagic fevers?

Abstract

There are many viruses known to cause viral hemorrhagic fevers in humans. The mechanisms causing hemorrhage are likely to vary among viruses. Some viruses, such as Marburg virus, are directly cytopathic to infected endothelial cells, suggesting infection of endothelial cells alone can cause hemorrhage. On the other hand, there are viruses which infect endothelial cells without causing any cytopathic effects, suggesting the involvement of host immune responses in developing hemorrhage. Typical examples of these include viruses of the hantavirus species. We hypothesize that impairment of endothelial cell's defense mechanisms against cytotoxic CD8$^+$ T cells is the mechanism of capillary leakage in hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome, which may be common to other viral hemorrhagic fevers. CD8$^+$ T cells may be a potential target for therapy of some viral hemorrhagic fevers.

Keywords: Hantavirus; Endothelial cells; Cytotoxic T lymphocytes; PD-1; PD-L1; PD-L2; Hantavirus pulmonary syndrome; Hemorrhagic fever with renal syndrome

There are many viruses known to cause viral hemorrhagic fevers in humans [1]. The mechanisms causing hemorrhage are likely to vary among viruses [2]. Some viruses, such as Marburg virus, are directly cytopathic to infected endothelial cells [3], suggesting infection of endothelial cells alone can cause hemorrhage. On the other hand, there are viruses which infect endothelial cells without causing any cytopathic effects, suggesting the involvement of host immune responses in developing hemorrhage. Typical examples of these include hantaviruses [4–7], which belong to genus *Hantavirus*, family *Bunyaviridae*.

Hantaviruses are RNA viruses possessing a segmented negative-stranded RNA genome [8,9]. Hantaviruses are conventionally divided into the Old World and the New World hantaviruses based on the geographic regions where they occur, although phylogenetic tree based on the genomic RNA sequences forms three main groups, Hantaan virus-like viruses, Puumala virus-like viruses and Sin Nombre virus (SNV)-like viruses [10]. The Old World hantaviruses, including Hantaan, Seoul, Dobrava and Puumala viruses which are seen throughout Europe and Asia, cause a human disease known as hemorrhagic fever with renal syndrome (HFRS) with more than 100,000 cases diagnosed annually. Clinically, HFRS is initially characterized by non-specific flu-like symptoms followed by thrombocytopenia, and a capillary leak syndrome with hemoconcentration. In severe cases renal failure and shock can develop. Mortality rates vary from approximately 1 to 15%, depending on the individual virus [11,12]. The New World hantaviruses include SNV and Andes virus, and are seen in North, Central, and South America [11,12]. While these hantaviruses have likely existed in the Americas for many years, they were recognized as a cause of disease when the first outbreak of hantavirus pulmonary syndrome (HPS) occurred in the southwestern United States in 1993 [13,14]. HPS shares many characteristics with HFRS, including thrombocytopenia and a capillary leak syndrome. However, there are some differences. The pathology seen with Old World hantaviruses focuses on the kidneys, but the major target organ for the New World hantaviruses is the lung. HPS cases progress to a severe degree more frequently than HFRS cases. After a prodromal phase similar to that of HFRS, patients very rapidly develop pulmonary edema, and shock, which often requires mechanical ventilation and/or extracorporeal membrane oxygenation [15], and the case fatality rate is approximately 35% (http://www.cdc.gov/ncidod/diseases/hanta/hps/noframes/caseinfo.htm). Thus, the New World hantaviruses cause some of the most lethal acute viral infections known, and antiviral therapy or vaccines are not yet available. In autopsied cases, most SNV antigens are found in endothelial cells, especially alveolar endothelial cells; but some other cells are also positive for viral antigen, such as monocyte/macrophages [16].

There have been three hypotheses to explain the mechanisms of increased capillary permeability. (1) The attack of infected endothelial cells by virus-specific cytotoxic T lymphocytes (CTLs); (2) tumor necrosis factor (TNF)-α production by infected monocyte/macrophages; and (3) the direct effect of viral infection on endothelial cell functions.

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Both in vivo and in vitro observations suggest that SNV is in general not directly cytopathic to infected cells including endothelial cells [6,7,16,17], although there are papers reporting apoptosis in some human and primate cell lines, such as human embryonic kidney 293 cells and African green monkey kidney Vero E6 cells, infected with hantaviruses [18–21]. β_3-Integrin is a cellular receptor for human-pathogenic hantaviruses [22,23]. Since the ligation of endothelial $\alpha_V\beta_3$-integrin increased transcapillary liquid flux [24], it was speculated that the virus–β_3-integrin interaction might be the mechanism of increased capillary permeability [22,23]. Infection of endothelial cells alone, however, failed to increase their permeability [6,7,25]. Hantavirus infection did inhibit β_3-integrin-directed migration of endothelial cells, which might contribute to hantavirus pathogenesis [26].

Mechanisms involving CTLs have been suggested by us [27–29] and others [30–32]. Lung tissues obtained at necropsy from HPS patients contain abundant large immunoblasts consisting of $CD4^+$ and $CD8^+$ T cells [16] [17], and high numbers of cytokine-producing cells including TNF-α, interleukine-2, and interferon (IFN)-γ [33], which could mediate capillary leakage. In addition, preliminary evidence suggests that, in SNV infection, the HLA-B*3501 allele is associated with increased risk for developing severe HPS, implying involvement of $CD8^+$ T cells [34]. We demonstrated very high frequencies of SNV-specific $CD8^+$ T cells detected by MHC class I/peptide tetramer staining in HPS patients' blood during acute illness, and the magnitude of virus-specific T cell responses was significantly higher in the patients with clinically severe HPS (patients who met clinical criteria requiring mechanical ventilation) than in patients with moderate disease (hospitalized but not requiring mechanical ventilation) [29]. We also showed that specific CTL recognized and increased the permeability of an immortalized HLA-matched human endothelial cell monolayer infected with SNV in transwell permeability assays [35]. These data suggest that SNV-specific $CD8^+$ T cells contribute to the observed capillary leakage during HPS. Since infected lungs at autopsy had no obvious damage in endothelial cells [16,17], capillary leakage is more likely to be caused by cytokine release than by endothelial cell lysis. Lysis of a small percentage of endothelial cells, which is difficult to detect in tissue sections, may be enough to cause capillary leakage, although bleeding is very rare in HPS.

A linkage between disease severity and MHC haplotype was also observed in patients with nephropathia epidemica (NE), a milder form of HFRS caused by Puumala virus infection. HLA-B8-DR3 extended haplotype was associated with severe outcome of the disease [36,37], and HLA-B27 was associated with milder disease [38], implying involvement of $CD8^+$ T cells in NE pathogenesis. In NE patients the kidney biopsies showed interstitial infiltration of lymphocytes, plasma cells, monocytes/macrophages, and polymorphonuclear leukocytes. An increased expression of cytokines including TNF-α and endothelial adhesion molecules was observed [39]. Plasma TNF-α levels were also elevated [40], and urinary excretion of interleukin-6 correlates with proteinuria [41].

In laboratory mice infected with Hantaan virus, virus-specific $CD8^+$ T cells, not neutralizing antibodies, were important for clearance of the virus [42–44]. These laboratory mice, as well as natural rodent reservoirs of hantaviruses, do not develop any disease similar to HPS or HFRS, suggesting in mice these virus-specific T cell are protective, not immunopathogenic. It is not understood why these rodents do not develop the disease. We should remember that there are many differences in the immune systems of humans and mice [45].

In transgenic mouse model of influenza infection, in which lung alveolar epithelial cells expressed influenza A virus hemagglutinin (HA), adoptive transfer of HA-specific $CD8^+$ T cells into the HA-transgenic mice induced lung injury, which was mediated by TNF-α produced by the HA-specific $CD8^+$ T cells and chemokines produced by alveolar epithelial cells attacked by the HA-specific $CD8^+$ T cells [46,47]. A similar mechanism may occur as a result of endothelial cell–$CD8^+$ T cell interactions.

A series of experiments performed by transplantation immunologists, however, showed that, contrary to lung alveolar epithelial cells, endothelial cells were protected from humoral and cellular immune responses in laboratory mice [48,49]. Johnson et al. analyzed humoral and cellular immune responses against β-galactosidase (BG) protein expressed in the endothelial cells of transgenic mice. In theory immune responses against BG protein would not be induced in BG-transgenic mice in which BG should be tolerated. Infection with recombinant vaccinia virus encoding BG, however, induced humoral and cellular immune responses in the BG-transgenic mice at the same level as responses in wild type FVB mice (from which the BG-transgenic were generated), and, more surprisingly, these infected mice remained healthy. No damage was observed in endothelial cells. The BG-transgenic mice also remained healthy after primed spleen cells or lymph node cells from immunized, wild type FVB mice, which contained $CD8^+$ (and $CD4^+$) T cells reacting to BG protein, were adoptively transferred into them. These results are surprising, but, nevertheless consistent with the down-regulation of $CD8^+$ T cell activation and cytolysis by PD-L (PD-1 ligand) 1 and PD-L2 molecules expressed on IFN-γ-activated endothelial cells both in humans (in vitro study) and mice [50–52] ($CD8^+$ T cells express programmed death-1 (PD-1) molecule).

To reconcile these two findings, the immunopathological mechanisms we would like to propose are:

(1) In HPS and HFRS patients the mechanisms to down-regulate $CD8^+$ T cell activation and cytolysis for protection of endothelial cells may be overwhelmed by the excess amount of activated $CD8^+$ T cells, which appears to occur in HPS [29].
(2) The protecting mechanisms may not be functioning properly because of the infection of endothelial cells. Glycoproteins of human-pathogenic hantaviruses have been found to have immunomodulatory functions [53,54], although the effects of hantavirus infection on PD-1, PD-L1 or PD-L2 are not known. It is also not known whether T cells are infected with hantavirus in vivo.

These two mechanisms can work synergistically, and infected monocyte/macrophages also can contribute to the