Hantavirus-induced pathogenesis in mice with a humanized immune system

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Hantaviruses are emerging zoonotic pathogens that can cause severe disease in humans. Clinical observations suggest that human immune components contribute to hantavirus-induced pathology. To address this issue we generated mice with a humanized immune system. Hantavirus infection of these animals resulted in systemic infection associated with weight loss, decreased activity, ruffled fur and inflammatory infiltrates of lung tissue. Intriguingly, after infection, humanized mice harbouring human leukocyte antigen (HLA) class I-restricted human CD8+ T cells started to lose weight earlier (day 10) than HLA class I-negative humanized mice (day 15). Moreover, in these mice the number of human platelets dropped by 77% whereas the number of murine platelets did not change, illustrating how differences between rodent and human haematolymphoid systems may contribute to disease development. To our knowledge this is the first description of a humanized mouse model of hantavirus infection, and our results indicate a role for human immune cells in hantaviral pathogenesis.

Hantaviruses (genus Hantavirus, family Bunyaviridae) are zoonotic pathogens that are transmitted to humans through inhalation of virus-containing aerosols (Jonsson et al., 2010). These are derived from excreta of persistently infected but asymptomatic rodents, their natural reservoir hosts. After infection with Eurasian hantavirus species such as Hantaan virus (HTNV), humans develop haemorrhagic fever with renal syndrome (HFRS). In contrast, hantavirus species in the Americas such as Andes virus (ANDV) cause hantavirus cardiopulmonary syndrome (HCPS). HFRS and HCPS are pathogenically closely related, as kidney failure and cardiopulmonary dysfunction are observed in both clinical syndromes although to different extents (Clement et al., 2014).

As there is no treatment available for hantavirus infection, we urgently need to understand the mechanisms underlying hantavirus-induced pathogenesis in order to develop suitable vaccines or therapeutics (Krüger et al., 2011). Besides the direct effects of hantavirus infection on endothelial cell barrier functions (Mackow & Gavrilovskaya, 2009), clinical observations suggest that CD8+ T lymphocytes play a pathogenic role in humans, as discussed in many reviews (Borges et al., 2006; Cosgriff, 1991; Kanerva et al., 1998; Khaiboullina & St Jeor, 2002; Peters et al., 1999; Schönrich et al., 2008; Terajima & Ennis, 2011). For obvious reasons, the latter concept is difficult to prove in vivo. In the Syrian hamster model of ANDV-induced HCPS, CD8+ T lymphocytes do not affect disease outcome (Hammerbeck & Hooper, 2011; Prescott et al., 2013).

The immune systems of rodents and humans have diverged for millions of years, driven by exposure to different...
Fig. 1. Hantavirus-induced symptoms in humanized mice. At 13 weeks after engraftment, hNSG mice and hNSG/HLA-A2 mice were infected with HTNV (10^5 f.f.u. i.p.) or left uninfected. (a) The viral load was determined by qRT-PCR in sera on a weekly basis (left graph) and in organs 3 weeks post-infection (p.i.) (right graph). It is shown as copies (ng total RNA)^{-1}. For this purpose, the viral copy number was relativized to the total amount of RNA. Error bars represent the mean ± SE (n = 8). (b) The body weight of hNSG mice (uninfected: n = 12; infected: n = 8) and hNSG/HLA-A2 mice (uninfected: n = 8; infected: n = 8) was monitored daily and is shown as percentage of initial body weight ± SEM (*, P < 0.05; **, P < 0.01; ***, P < 0.001; Mann–Whitney test).
spectra of pathogens, and this has resulted in functional differences (Mestas & Hughes, 2004). In order to explore the role of human CD8+ T lymphocytes in hantavirus-induced pathology, we generated mice with a humanized immune system using an established protocol based on NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ mice, or in short NSG mice (Kim et al., 2012). NSG mice and transgenic NSG mice expressing human leukocyte antigen (HLA)-A2 (NSG/HLA-A2 mice) were purchased from the Jackson Laboratory. They were inoculated with human hematopoietic stem cells (HSCs) that had been isolated from umbilical cord blood. NSG/HLA-A2 mice were humanized with HLA-A2-matched HSCs (hNSG/HLA-A2 mice) which facilitates the development of functional human CD8+ T cells, as previously shown (Billerbeck et al., 2013; Jaiswal et al., 2009; Shultz et al., 2010; Strowig et al., 2009). In contrast, parental NSG mice reconstituted with human HSCs (hNSG mice) have no functional human CD8+ T cells, due to the lack of human HLA class I molecules. At 11 weeks post-reconstitution, the extent of engraftment was validated by cytofluorimetric analysis of peripheral blood mononuclear cells derived from humanized mice (Figs S1–S3). Successfully engrafted mice were distributed into various groups based on engraftment levels, and infected intraperitoneally (i.p.) with 10⁶ f.f.u. of HTNV (strain 76-118).

Using an established reverse transcription quantitative real-time PCR (qRT-PCR) (Kramski et al., 2007), the hantaviral genome was clearly detectable in sera and organs of infected hNSG/HLA-A2 mice, with the highest numbers of viral genomes found in the lung (Figs 1a and S4). Similar viral loads were found in sera and organs of hNSG mice (Fig. S5). There were no significant differences in viral replication between the two models. Intriguingly, despite similar viral loads, hNSG/HLA-A2 mice showed statistically significant earlier and more progressive weight loss than hNSG mice (Fig. 1b). In contrast, there was only a slight but not rapidly progressing weight loss in HTNV-infected parental NSG mice within the observation period (Fig. S6). Moreover, HTNV-infected hNSG/HLA-A2 mice developed severe clinical signs such as ruffled fur, hunched posture and
reduced activity. They became terminally ill and required euthanization faster than HTNV-infected hNSG mice (Fig. 1c). Histopathological analysis of lung tissue from hantavirus-infected humanized mice revealed mild to moderate interstitial lung infiltrates similar to hantavirus-induced human disease (Clement et al., 2014). In the alveolar walls of lung tissue from HTNV-infected hNSG/HLA-A2 mice, at least twice as much inflammatory cell infiltration was detected as compared to hNSG mice (Fig. 1d, e). In contrast, inflammatory cell infiltration was not detected in the lungs of uninfected mice.

Collectively, our data indicate that functional human CD8+ T cells accelerate and aggravate hantavirus-induced disease. In accordance, after adoptive transfer of murine T cells in the SCID mouse model of hantavirus infection, pulmonary oedema occurred earlier than in non-transferred HTNV-infected mice (Koma et al., 2014). It is likely that CD8+ T cells contribute to hantavirus-associated immunopathology by mechanisms other than cytotoxic granule-mediated apoptosis, as hantavirus nucleocapsid protein interferes with the enzymic activity of both granzyme B and caspase (Gupta et al., 2013). It is possible that CD8+ T cells interact with hantavirus-infected endothelial cells and increase the microvascular leakage directly, by releasing TNF-α as reported for neutrophils (Finsterbusch et al., 2014). They could also act indirectly by modulating activation of other immune cells such as neutrophils, which have been shown to play a pivotal role in hantavirus-induced disease (Koma et al., 2014; Raftery et al., 2014).

Besides pneumonia, a drop in platelet count is another hallmark of hantavirus-associated disease. Accordingly, by using cytofluorimetric analysis we determined whether hantavirus affects platelet numbers in humanized mice. Platelets are produced and released into the circulation by megakaryocytes (Schulze & Shviddasani, 2005). In peripheral blood from uninfected hNSG and hNAG/HLA-A2 mice, we found (in addition to murine platelets) low numbers of human platelets as previously described (Ishikawa et al., 2005). Upon hantavirus infection, the number of murine platelets remained unchanged in both hNSG (data not shown) and hNAG/HLA-A2 mice (Fig. 2a). In striking contrast, at 14 days post-infection (p.i.), the number of human platelets dropped significantly by 77% in hNSG/HLA-A2 mice (Figs 2b and 3), whereas in hNSG mice the reduction was non-significant (data not shown).

Thus, in humanized mice, hantavirus rapidly and selectively induces a drop of human platelet counts. In principle, this could be due to elimination of hantavirus-infected human megakaryocytes by cytotoxic T cells and a subsequent decrease in human platelet production. In line with this view, human megakaryocytic cells are susceptible to hantavirus infection and upregulate HLA class I molecules in response to hantavirus infection (Lütteke et al., 2010).

On the other hand, loss of platelets could also be due to increased elimination of platelets. It is known that hantaviruses bind to human platelets via β3 integrin (Gavrilovskaya et al., 2010). Thus, human platelets coated with hantaviruses may be bridged through integrins to phagocytic cells such as macrophages or neutrophils, being subsequently phagocytosed. Indeed, there is evidence that neutrophils can clear activated platelets from the circulation (Manfredi et al., 2010). In contrast, hantaviruses do not bind to murine β3 integrin (Raymond et al., 2005) and therefore do not coat murine platelets, explaining why in our experiments murine platelets were not affected. However, whether or not the drop in platelet count we observed is directly linked to the lethality and weight loss seen in our model is unclear, due to the relatively small number of human platelets and the presence of large numbers of functional murine platelets.

To our knowledge, this is the first report of a humanized mouse model of hantavirus infection. It demonstrates the role of immunopathology in human hantavirus disease, as opposed to purely rodent models. In the future, this small animal model will help to assess the virulence of newly discovered hantaviruses, or previously discovered hantaviruses with unclear pathogenic potential. Moreover, it will be a
valuable tool for testing candidate vaccines, antiviral drugs or novel treatment strategies preventing deleterious human immune response against hantavirus.

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