The neonatal Fc receptor does not modulate hepatitis C virus neutralization

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The human neonatal Fc receptor (FcRn) is the only receptor known to be able to transport IgG across cell barriers (Roopenian & Akilesh, 2007). During the perinatal period, it provides the neonate with humoral immunity, by transporting maternal IgG across the placenta. Throughout life, it mediates transcytosis, thereby ensuring the extravascular biodistribution of IgG. In addition to this transcytotic function, FcRn plays a critical role in serum IgG homeostasis, through the recycling of IgG away from a catabolic pathway in the vascular endothelium, thereby extending the lifespan of IgG in the bloodstream. FcRn binds IgG in acidic conditions and releases IgG in neutral or basic conditions (Raghavan et al., 1995). In most cell types, FcRn resides principally in early acidic endosomal vesicles, in which it binds to IgG entering the cell by endocytosis and then recycles IgG back to the plasma membrane, where the neutral pH of the extracellular compartment causes the IgG to be released from the FcRn (Tesar et al., 2006).

It has recently been demonstrated that influenza A virus (IAV) neutralization is modulated by the FcRn-dependent transport of antibodies to the endosomal compartment (Bai et al., 2011). The application of a pH-dependent mAb (clone Y8) to the basolateral surface of Madin–Darby canine kidney (MDCK) cells expressing rat FcRn significantly decreased virus replication rates following apical exposure of the cell monolayer to influenza virus. More importantly, prophylactic administration of the Y8 mAb before viral challenge resulted in significantly lower pulmonary virus titres in WT mice but not in FcRn-knockout (FcRn-KO) mice. It has been suggested that the Y8 mAb neutralizes the virus intracellularly, as it can block fusion and egress from endosomes, resulting in the transport of virions to the lysosome for degradation. It has been suggested that other IgG antibodies with a broad spectrum of action or directed against the viral envelope regions containing the fusion domain might work in a similar manner, or even more effectively, through FcRn-dependent intracellular neutralization mechanisms.

FcRn is produced in hepatocytes (Schilling et al., 2003), and the most efficient neutralizing mAbs against hepatitis C virus (HCV) have been shown to inhibit infection at a post-attachment step (Sabo et al., 2011). We therefore investigated the possible involvement of an FcRn-dependent mechanism in hepatitis C virus (HCV) neutralization. Our study, in both HCV pseudoparticles and HCV in cell-culture models, showed that FcRn was not involved in the intracellular neutralization of HCV, in contrast to the situation observed for influenza A virus.

One supplementary figure is available with the online Supplementary Material.
was observed in this clone in Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) experiments (Fig. 1a) and on Western blot analysis (Fig. 1b). This FcRn-depleted Huh7.5 clone was compared with Huh7.5 cells expressing a control shRNA in HCV neutralization assays in vitro. We first investigated the impact of FcRn depletion on HCV neutralization with the HCV pseudoparticle model (HCVpp), which constitutes a relevant model for studies of the early phases of HCV entry (Bartosch et al., 2003). The activity of an AR3A mAb, targeting a discontinuous epitope in the envelope glycoprotein E2 and known to inhibit HCV at both pre- and post-attachment stages (Giang et al., 2012), was assessed, together with that of a serum previously shown to neutralize HCV (from a patient chronically infected with HCV genotype 1b; data not shown). The interaction between AR3A mAb and the FcRn at an acidic pH was checked using flow cytometry as shown in the Fig. S1 (available in the online Supplementary Material). Activity was assessed against viral particles pseudotyped with HCV envelope glycoproteins derived from genotypes 1a (H77) and 2a (JFH-1). No difference between the FcRn-depleted and control cells was observed. The AR3A mAb and the HCV-1b-infected serum decreased HCVpp entry by 90 and 60 %, respectively, in both cell types (Fig. 2). We explored the potential role of FcRn in HCV neutralization further, by performing additional assays in the HCV cell-culture (HCVcc) (JFH-1) model, mimicking all the steps of the HCV infectious cycle. Previous studies have reported that the most potent HCV neutralizing antibodies inhibit infection at a post-attachment step (Sabo et al., 2011), so the HCVcc model may be a more relevant system for addressing the question posed here, as it reconstitutes the various stages of HCV entry, including envelope fusion and virion uncoating (Lohmann & Bartenschlager, 2014). We therefore used the AR3A mAb and two serum samples from chronically infected patients (with genotypes 1b and 4, respectively) to determine whether the FcRn exerted neutralizing effects preferentially during the late phases of HCVcc entry. In tests with HCVcc used at an m.o.i. of 0.01, no difference was observed between the FcRn-depleted and control cells, with the AR3A mAb and human serum samples decreasing HCVcc infection by 100 and 70–80 %, respectively, in both cell types (Fig. 3a). To avoid a bias induced by a possible antibody-mediated neutralization at a very early entry step(s), similar experiments were conducted after virions were bound to the cells at 4 °C to check the neutralization of the infection at a post-attachment step (Fig. 3b). The results displayed the same tendency as for the experiments conducted pre-attachment. No difference in terms of neutralizing activity could be observed between the FcRn-depleted and control cells (Fig. 3).

In the IAV model, the critical step at which the Y8 mAb interferes with the virus life cycle during its trafficking by FcRn is unknown (Bai et al., 2011). It is not clear whether FcRn organizes the antibodies in the endosome into an
orientation facilitating interactions with the viral envelope or whether it simply increases the endosomal concentration of the antibodies to levels more effective at blocking virus fusion. However, FcRn is involved in intracellular virus neutralization, as demonstrated by the findings for cell and KO mouse models (Bai et al., 2011). Unfortunately, it is not possible to use an FcRn-KO mouse model for investigations of the potential FcRn-dependent effect on
HCV neutralization, as these mice are not susceptible to HCV infection. Alternative approaches based on the genetically humanized mouse model for HCV infection could be established (Dorner et al., 2011), but this would require a back-cross of this mouse model with FcRn-KO mice. However, our results for a clone of the HCV-susceptible Huh7.5 cell line displaying strong FcRn depletion demonstrated that FcRn plays no role in HCV neutralization in vitro.

In conclusion, despite expression of the FcRn in hepatocytes and the occurrence of FcRn-dependent neutralization mechanisms in other viral models (Bai et al., 2011), we have shown here that FcRn does not modulate HCV neutralization in vitro. These results indicate the relative impact of intracellular FcRn-dependent neutralization in different viral models, and may be useful for the future development of antibody-based treatments for HCV infection (Sautto et al., 2012).

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### References


