HEPATOLOGY

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Journal:	*Hepatology*
Manuscript ID	HEP-17-1212
Wiley - Manuscript type:	Hepatology Elsewhere
Date Submitted by the Author:	22-Jun-2017
Complete List of Authors:	Ober, Raimund; Texas A&M University Health Science Center Ward, Sally; Texas A&M University College of Medicine
Keywords:	

Hepatic function of FcRn revealed: implications for overcoming drug-mediated hepatotoxicity

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Hepatocytes represent the most abundant cell type in the liver and are responsible for multiple activities, including their crucial function as the site of albumin biosynthesis. To date, although it is known that albumin is a ligand of the neonatal Fc receptor, FcRn, how the expression of this receptor in hepatocytes regulates albumin homeostasis is poorly understood. A recent investigation by Blumberg and colleagues (Pyzik M, Rath T, Kuo TT, Win S, Baker K, Hubbard JJ, Grenha R, et al. Hepatic FcRn regulates albumin homeostasis and susceptibility to liver injury. Proc Natl Acad Sci U S A 2017;114:E2862-E2871) addresses this issue. This study demonstrates that FcRn transports albumin away from the biliary tract, thereby maintaining circulating albumin levels. Further, FcRn deletion or inhibition leads to increased albumin accumulation in the bile, combined with higher intracellular albumin levels in hepatocytes, which can be exploited to reduce the hepatotoxic effects of the analgesic, acetaminophen. These analyses solve a longstanding open question in FcRn biology and also have broad relevance to the treatment of drug-mediated hepatotoxicity.

The neonatal Fc receptor, FcRn, regulates the levels and transport of immunoglobulin G (IgG) and albumin in the body (1, 2). Despite the designation of FcRn as the *neonatal* Fc receptor due to its initial identification as the transporter of maternal IgG across the neonatal rodent gut, this receptor is ubiquitously expressed in both parenchymal and hematopoietic cells throughout life. FcRn interacts with IgG and albumin at acidic, endosomal pH with an affinity that becomes progressively lower as near neutral pH is approached. This pH-dependent binding allows FcRn to bind to ligand in early endosomes following internalization into cells. Endosomal binding is followed by FcRn-mediated recycling or transcytosis and exocytic release (3, 4). The cell biological processes involved in endocytic sorting and recycling/transcytosis of IgG have been extensively studied in endothelial and epithelial cells (1, 2). It is also known that both hematopoietic and endothelial cells contribute to IgG and albumin homeostasis (5, 6).

Although FcRn expression was identified in hepatocytes in 1995 by Blumberg and colleagues (7), the function of this receptor in this vitally important cell type has to date not been elucidated. Hepatocytes are not only the most prevalent cell type in liver, but are also the site of albumin biosynthesis. A recent study led by the Blumberg laboratory has addressed the longstanding, unresolved issue concerning the role of hepatocytic FcRn. In addition to providing novel insight into FcRn activity in hepatocytes, this investigation demonstrates that hepatotoxicity resulting from overdoses of the analgesic, acetaminophen (para-acetylaminophenol, APAP), can be ameliorated by targeting FcRn (8). Given reports that over 100,000 cases of acetaminophen toxicity occur annually in the US alone, the significance of these observations cannot be overstated.

Blumberg and colleagues use a variety of approaches in an experimental tour de force to characterize the function of FcRn in hepatocytes. Through site-specific deletion of this receptor in hepatocytes in mice, they demonstrate that FcRn loss results in reduced serum, but increased biliary, levels of albumin. Interestingly, these effects are not observed for IgG, demonstrating that hepatocytic FcRn deficiency has differential effects on IgG and albumin (a possible area for future investigation). *In vitro* studies with FcRn/albumin-expressing MDCK cells, to model hepatocyte functions in albumin biosynthesis and FcRn-mediated transport, show that FcRn recycles albumin towards the basal surface (equivalent to the sinusoidal surface of a hepatocyte) and transcytoses albumin from the apical (canalicular) to basal surface. These analyses provide cell biological insight into the activity of FcRn as a gatekeeper to maintain circulating albumin levels that, if perturbed by FcRn ablation, results in increased delivery of albumin into the biliary tract. A related outcome of these analyses, discussed further below, is the observation that newly synthesized albumin is present at higher levels within FcRn-deficient hepatocytes and albumin-expressing MDCK cells. This leads to the interesting possibility that FcRn contributes to secretion of this protein during biosynthesis.

Albumin has many diverse properties: it is a major regulator of intravascular oncotic pressure and, as a free radical scavenger, can protect against oxidative stress. In addition, through its ability to promiscuously bind and transport multiple substrates such as hormones, ions, proteins and water, combined with its prolonged *in vivo* persistence, this protein serves an important carrier function in the body. The targeting of albumin to engineer longer-lived therapeutics represents an area of high activity in biopharma. However, the transport function of albumin can also include drugs/toxins, with the undesirable consequence that albumin binding can extend the

in vivo half-life of such molecules. The commonly used analgesic, APAP, is an example of a drug that binds to albumin. The corollary of the half-life extending, or buffering, effect is that strategies to enhance albumin clearance could protect against APAP-mediated toxicity. That this is indeed the case has been clearly demonstrated by Blumberg and colleagues: first, FcRn-deficient mice exhibit greater resistance to lethal doses of APAP compared with the corresponding wild type mice. Second, the delivery of antibodies or peptides that inhibit FcRn-albumin interactions, thereby resulting in increased transport of albumin into bile and hypoalbuminemia, protects against APAP toxicity.

However, as the authors point out, only about 10% of therapeutic doses of APAP are bound by albumin. This raises the question as to whether additional mechanisms of protection beyond increased albumin excretion are operative to alleviate APAP toxicity in the face of FcRn deficiency or inhibition. This is where the observation of increased levels of intracellular albumin accumulation in hepatocytes in the absence of functional FcRn also comes into play through the anti-oxidant activity of albumin. APAP-mediated toxicity towards hepatocytes involves oxidative stress. Consequently, higher albumin concentrations in FcRn-deficient hepatocytes endows these cells with enhanced resilience against the elevated levels of reactive oxygen species (ROS) resulting from exposure to toxic doses of APAP. Thus, both increased excretion of APAP/albumin complexes and enhanced protection of hepatocytes against ROS contribute to the beneficial effects of FcRn insufficiency following ingestion of toxic levels of APAP, although the relative contributions of each pathway remain to be determined. In this context, low to undetectable levels of FcRn expression in tumor cells result in increased

intracellular albumin levels (9), leading to the speculation that these elevated albumin concentrations may also confer increased tolerance of malignant cells to ROS.

The use of FcRn inhibitors to clear albumin-bound drugs such as APAP raises questions concerning the side effects of the hypoalbuminemia that is induced. Despite the essential functions of albumin, these concerns can be countered by the following: first, the effects of high affinity FcRn inhibitors such as peptides or antibodies are likely to be relatively short-lived due to the rapid clearance of this class of therapeutics (1). Second, albumin biosynthesis by hepatocytes is highly sensitive to albumin levels, and compensatory upregulation occurs in response to increased degradation of this protein.

Collectively, these studies provide interesting insight into a novel function of FcRn that is of considerable therapeutic relevance. In general, the beneficial effects of albumin greatly outweigh its ability to buffer toxic molecules. Nevertheless, the identification of potent inhibitors of FcRn that negate this buffering capacity, combined with the increase in mechanistic understanding of FcRn-albumin biology in hepatocytes that result from this recent study, provide an exciting path for the treatment of APAP toxicity that could have broad implications for the management of adverse exposure to other drugs and toxins.

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