cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

EDITORIAL

Does Massive Antigen Burden Allow Hepatic Viruses to Induce Regulatory T Cells and Their Tolerance and Persistence?

Regulatory T cells (Tregs), typically expressing CD25 and the transcription factor forkhead box P3 (FoxP3)—which suppress effector T-cells via contact-dependent mechanisms and immunosuppressive cytokines such as interleukin 10 (IL-10) and transforming growth factor β (TGFβ)—play a critical role in the establishment of chronic hepatitis infections in humans.[1] Although primate studies have contributed significantly to our understanding of innate and adaptive responses, and have shown that Tregs are induced in both resolving and persistent viral hepatitis infection, understanding the mechanisms by which Tregs are themselves induced and then induce tolerance in vivo has been hampered by the absence of a convenient and authentic small-animal model of these infections.

An optimal model for dissecting the critical immunosuppressive processes most relevant during the acute and chronic phases of hepatitis B and C viral (HBV and HCV) infections would feature chronic infection by a highly productive, primarily hepatotropic virus (ideally strains of hepatitis C or B) in the absence of significant systemic immunodeficiency. Ideally, such a model would also be without germline defects in pattern-recognition receptor systems, have some capability for humanization, and not rely on potentially tolerogenic constitutive viral antigen expression in the absence of infection. Such a model does not yet exist. Until one is developed, we must glean an improved understanding of the hierarchy of immunosuppressive pathways relevant in chronic hepatotropic viral infections from creative but incomplete models.

Most liver-specific transgenic mouse models shed little light on host–virus interaction because of their lack of significant immunogenicity. One of the first transgenic models to overcome this barrier involved mice with floxed HCV structural core, E1, E2, and nonstructural NS2 constructs induced to have hepatocyte-specific expression by adenovirally delivered Cre recombinase.[2] In this model, a robust cytotoxic $CD8^+$ T-cell response against viral antigens resulted in massive T-cell infiltration into the liver and antigen clearance. When this model was recapitulated in relatively CD8 T-cell–deficient interferon regulatory factor-$1^{-/-}$ mice, transient hepatitis resulted, but the HCV-expressing hepatocytes were not cleared despite the persistence of virus-specific effector $CD8^+$ T cells. These early studies focused on the role of $CD8^+$ T cells play in hepatic inflammation, but they also suggested regulation of cytotoxic T cells in vivo by mechanisms unknown at that time.

Since that time, several mouse models of acute and chronic hepatitis have provided strong evidence that Tregs play a fundamental role in dampening the potentially deleterious impact of activated effector T cells. For instance, in the concanavalin A–induced mouse hepatitis model, which is a frequently used model of human autoimmune and viral hepatitis, the intrahepatic inflammatory cytokines produced by effector T cells appear to induce the accumulation of intrahepatic $CD4^+CD25^+$ regulatory T cells.[3] These Tregs, similar to human Tregs, express high levels of galectin-9, which in this model induces apoptosis of $CD4^+CD25^-$ effector T cells and attenuates liver inflammation. Due to the stochastic nature of self-antigen expression in the concanavalin A model, the role of antigen recognition and the immunoregulatory actions of specific viral proteins in the generation of Tregs cannot be assessed.

However, vector-based models have demonstrated that individual HCV proteins have a divergent impact on endogenous T-cell responses in immunocompetent mice. For example, when immunized with recombinant adenoviruses encoding either HCV Core or NS3, wild-type C57BL/6 mice developed NS3-specific T cells exhibiting typical effector cytokine and cytolytic responses, but generated HCV core-specific T cells that were hypoproliferative, predominantly IL-10-producing, and enriched for $CD4^+foxp3^+$ Tregs.[4] Although antigen-expression was not necessarily hepatocyte specific, and no chronic infection occurred, this model highlighted the potential role of HCV core protein itself in establishing immunosuppressive conditions thought likely to contribute to chronicity in humans.

Human liver/mouse chimeras offer the potential to study the immunosuppressive impact of human hepatotropic viruses in the mouse system, but to date this potential has not been fully realized for technical reasons. The human liver-urokinase plasminogen activator/severe combined immunodeficiency (uPA/SCID), nonobese diabetic/severe combined immunodeficiency (NOD/SCID), and fumarylacetoacetate hydrolase-recombination activating $gene^{-/-}$ γC^{null} (FAH-$Rag^{-/-}$ γC^{null}) models can support high-level human hepatocyte engraftment and can support HCV[5] or HBV[6] replication but are immunodeficient. The AFC8-hu HSC/Hep model, in which Balb/C $Rag2^{-/-}\gamma C^{null}$ mice are cotransplanted with both human hepatocyte progenitors and human $CD34^+$ hematopoietic stem cells (HSC), reconstituting the mouse with both human liver mass and a human immune system, can support HCV infection.[7] HCV infection in this model indeed produces liver inflammation, HCV-specific effector $CD4^+$ and $CD8^+$ T-cells, stellate cell activation, and fibrosis. However, only half of the mice successfully support HCV infection, human hepatic reconstitution in this model is modest (15%), and there is no detectable viremia. Furthermore, although T cells engraft well, the B-cell compartment does not similarly reconstitute, resulting in a potentially biased assessment of host immune responses.

Another human immune system reconstitution model involves human leukocyte antigen (HLA)-transgenic NOD/SCID/IL2R$^{-/-}$ mice reconstituted with CD34^{+} hematopoietic stem cells. When exposed to HCV nonstructural antigens via a nonproductive hepatotropic adenovirus, this model generates strong HCV-specific HLA-A2-restricted T-cell responses that mediate partial but not complete adenoviral clearance from the liver.[8] The authors of this study postulated that this incomplete clearance might be related to incomplete reconstitution of the $^{-/-}\gamma C^{null}$ NK and myeloid compartments in the model, but did not report on regulatory T-cell induction as an alternative explanation. The lack of viremia in this model also limits its authenticity. Thus, human liver chimeric models, while promising, have not yet explained the generation and function of hepatitis virus-specific Tregs in mice in vivo.

Lymphocytic choriomeningitis virus (LCMV), while not a hepacivirus or hepadnavirus, has features that make it attractive for modeling human chronic viral hepatitis. First, LCMV, while not liver specific, has strong hepatotropism. Certain LCMV strains typically result in acute infections whereas LCMV clone 13 results in a persistent infection. Clone 13 infections induce early impairment of LCMV-specific CD4^{+} T-cells with anergy, deviation of CD4^{+} T-cells toward IL-10^{+} production, and inhibition of LCMV-specific CD8^{+} T cells,[9] similar to processes reported in the transition from acute to chronic HCV infections in humans. In the setting of uncontrolled viral replication induced by macrophage depletion, LCMV-specific CD8^{+} T-cells can even induce liver fibrosis.[10]

In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Lapierre and colleagues[11] characterize the dynamics of LCMV-specific CD4^{+} and CD8^{+} T-cell responses in the presence or absence of constitutive expression of a single subdominant nucleoprotein epitope (NP) expressed using the transthyretin (TTR) promoter. When exposed to two typically resolving strains of LCMV, TTR-NP mice developed chronic infections. This appeared predominantly due to persistent intrahepatic virus and was associated with increased serum alanine aminotransferase (ALT) levels after 2 months and infiltration of effector CD4^{+} and CD8^{+} T-cell numbers into the liver. However, relative to T cells in wild-type mice, NP-specific CD4^{+} and CD8^{+} T cells were reduced and CD4^{+} and CD8^{+} T-cells overexpressed the inhibitory costimulation marker programmed death-1 (PD-1) and had impaired cytotoxic function, consistent with exhaustion after the early acute phase of infection. LCMV-infected TTR-NP mice also manifested high serum levels of the immunosuppressive cytokine IL-10 produced by a high number of CD4^{+}CD25^{+}foxp3^{+}Helios^{+} Tregs. The defect in NP-specific T-cell function was not due to liver-intrinsic mechanisms because NP-specific T-cells adoptively transferred into RAG-deficient TTR-NP that do not develop Tregs exhibited enhanced, not impaired, proliferation and cytokine function. Similarly, depletion of CD25^{+} cells using an anti-CD25 antibody led to decreased numbers of CD8^{+}PD-1^{+} cells, restoration of effector function, and a decrease of serum IL-10 and viral clearance. Critically, heterozygote TTR-NP/wild-type mice under similar conditions exhibited attenuated generation of TREGs and relatively normal NP-specific effector T-cell function, suggesting that antigen burden is the key factor driving Treg induction and subsequent suppression. Thus, the model indicates that high-level expression of a viral antigen in hepatocytes leads to the development of Tregs that interfere with CD4^{+} and CD8^{+} effector T-cell responses, impairing early viral clearance and promoting chronic infection.

The authors should be lauded for well-constructed experiments that address a critical gap in our understanding of the immunopathogenesis of chronic hepatic infections. The two most original features of this model are that 1) chronic liver damage is induced by polyclonal T-cells in the context of an active viral infection, and 2) antigen expression can be partially modulated. How authentically does this mouse model recapitulate human acute to chronic viral hepatitis? Unlike chronic viral hepatitis, viral antigen is expressed constitutively as a self-antigen in all hepatocytes. Thus, generation of peripheral tolerance to viral antigens could predate viral infection. It is unknown to what extent viral antigen expression in this model mimics antigen expression in human hepatocytes infected with HCV or HBV. Further, the critical link between early innate and adaptive immunity cannot be probed because LCMV, unlike HCV, does not directly interfere with RIG-I-like receptor signaling and early type I interferon induction.[12]

Despite these limitations, the work of Lapierre et al strongly supports a critical role for CD4^{+}foxp3^{+}IL-10^{+} Tregs in inducing antigen-specific T-cell tolerance and the establishment of chronic hepatic viral infection. These data, combined with a myriad of translational human ex vivo and in vitro studies, suggest that therapeutic manipulation of Tregs either via depletion or inactivation may be worth investigating as treatment for chronic hepatitis B virus and drug-resistant HCV infections.

DAVID E. KAPLAN
Division of Gastroenterology
Perelman School of Medicine
University of Pennsylvania
Philadelphia, Pennsylvania

Gastroenterology Section
Philadelphia VA Medical Center
Philadelphia, Pennsylvania

References

1. Knolle PA, Thimme R. Hepatic immune regulation and its involvement in viral hepatitis infection. Gastroenterology 2014;146:1193–1207.
2. Takaku S, Nakagawa Y, Shimizu M, et al. Induction of hepatic injury by hepatitis C virus-specific CD8^{+} murine cytotoxic T lymphocytes in transgenic mice expressing the viral structural genes. Biochem Biophys Res Commun 2003;301:330–337.
3. Ju Y, Shang X, Liu Z, et al. The Tim-3/galectin-9 pathway involves in the homeostasis of hepatic Tregs in a mouse

model of concanavalin A-induced hepatitis. Mol Immunol 2014;58:85–91.
4. Krishnadas DK, Ahn JS, Han J, et al. Immunomodulation by hepatitis C virus-derived proteins: targeting human dendritic cells by multiple mechanisms. Int Immunol 2010;22:491–502.
5. Mercer DF, Schiller DE, Elliott JF, et al. Hepatitis C virus replication in mice with chimeric human livers. Nat Med 2001;7:927–933.
6. Dandri M, Burda MR, Torok E, et al. Repopulation of mouse liver with human hepatocytes and in vivo infection with hepatitis B virus. Hepatology 2001;33:981–988.
7. Washburn ML, Bility MT, Zhang L, et al. A humanized mouse model to study hepatitis C virus infection, immune response, and liver disease. Gastroenterology 2011;140:1334–1344.
8. Billerbeck E, de Jong Y, Dorner M, et al. Animal models for hepatitis C. Curr Top Microbiol Immunol 2013;369:49–86.
9. Brooks DG, Teyton L, Oldstone MB, et al. Intrinsic functional dysregulation of CD4 T cells occurs rapidly following persistent viral infection. J Virol 2005;79:10514–10527.
10. Lang PA, Recher M, Honke N, et al. Tissue macrophages suppress viral replication and prevent severe immunopathology in an interferon-I-dependent manner in mice. Hepatology 2010;52:25–32.
11. Lapierre P, Janelle V, Langlois MP, et al. Expression of viral antigen by the liver leads to chronic infection through the generation of regulatory T cells. Cell Mol Gastroenterol Hepatol 2015;1:325–341.
12. Clingan JM, Ostrow K, Hosiawa KA, et al. Differential roles for RIG-I-like receptors and nucleic acid-sensing TLR pathways in controlling a chronic viral infection. J Immunol 2012;188:4432–4440.

Correspondence
Address correspondence to: David E. Kaplan, MD, MSc, FACP, University of Pennsylvania, Perelman School of Medicine, Division of Gastroenterology, 415 Curie Boulevard, BRB II/III 9th Floor, Philadelphia, Pennsylvania 19104. e-mail: dakaplan@mail.med.upenn.edu.

Conflicts of interest
The author discloses no conflicts.

2352-345X
http://dx.doi.org/10.1016/j.jcmgh.2015.03.004