HCV adaptations to altered CD8⁺ T-cell immunity during pregnancy

Aryn A Price^{1,2,3}, Arash Grakoui^{2,3,4} & Jonathan R Honegger^{*,5,6}

Robust, polyfunctional CD8⁺ T-cell responses targeted to multiple viral epitopes have been associated with successful resolution of acute HCV infection. In most individuals, however, immunity fails and chronic viremia ensues as CD8⁺ T cells become functionally exhausted or select viral variants with escape mutations in class I epitopes. During pregnancy, dysfunctional HCV-specific CD8⁺ T-cell responses may be further impaired by maternal–fetal tolerance processes such as the expansion of regulatory T-cell populations or hormonal changes. Recent evidence suggests that HCV adapts to relaxed maternal CD8⁺ T-cell pressure in pregnancy by shedding unfit escape mutations in certain class I epitopes, resulting in the selection of viruses with more efficient replication. Emergence of HCV variants with enhanced replicative capacity during pregnancy has ramifications for mother-to-child transmission, the primary route of HCV infection in children, and could potentially be relevant to

other important, vertically transmissible, persistent viruses such as HBV and HIV.

HCV has infected over 185 million people worldwide and persists in approximately 75% of these individuals, predisposing to liver inflammation, cirrhosis and hepatocellular carcinoma [1]. More than 1 million children are born to HCV-infected mothers each year, and 3–5% acquire the infection *in utero* or at delivery, making vertical transmission the leading route of pediatric HCV infection in the developed world [2]. Here, we review the critical role of CD8⁺ T-cell immunity in HCV infection, its modulation in pregnancy and recent evidence that HCV takes advantage of the unique immunologic niche of pregnancy to improve replicative fitness and potentially promote vertical transmission.

Exhausted CD8⁺ T-cell immunity in chronic HCV

CD8⁺ T-cell immunity is vital for control of HCV infection but fails in most

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¹Department of Microbiology & Immunology, Microbiology & Molecular Genetics Program, Emory University, Atlanta, GA, USA

²Emory Vaccine Center, Emory University, Atlanta, GA, USA

³Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA

⁴Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, GA, USA

⁵Center for Vaccines & Immunity, Nationwide Children's Hospital, Columbus, OH, USA

⁶Department of Pediatrics, The Ohio State University School of Medicine, Columbus, OH, USA

*Author for correspondence: Tel.: +1 614 355 2873; jonathan.honegger@nationwidechildrens.org

individuals. When acute HCV infection is controlled successfully, the effective immune response predictably includes expansion of highly functional HCV-specific CD8⁺ T cells targeted to multiple viral epitopes. These cell populations secrete antiviral cytokines, such as IFN- γ , TNF- α and IL-2, and exert cytotoxic activity that is sustained until viremia resolves. In the more common scenario of progression to chronic infection, HCV-specific CD8⁺ T-cell responses are variable in the acute phase, but inevitably develop functional exhaustion or select viral variants that escape T-cell recognition [3]. Exhausted HCV-specific CD8⁺ T cells exhibit poor cytotoxicity, cytokine secretion and proliferative capacity, and express high levels of inhibitory molecules, such as programmed cell death 1 and low levels of self-renewal markers, such as the IL-7 receptor α -chain CD127 [4]. Numerous mechanisms have been proposed to explain CD8⁺ T-cell dysfunction in chronic HCV, including defective antigen presentation, loss of CD4⁺ T-cell help, and intrahepatic enrichment of T regulatory cells (Tregs) [3]. In the chronic phase of infection, HCV-specific CD8⁺ T cells are often difficult to detect in the peripheral blood but are maintained in the liver. Whether exhausted cells exert any antiviral activity *in vivo*, particularly within the liver, and can be rescued from an exhausted state, are questions of intense interest. Notably, peripheral HCV-specific CD8⁺ T cells targeting epitopes that escaped recognition express lower levels of inhibitory coreceptors than those cells targeting intact epitopes, suggesting that there may be differences in T-cell effector function depending on viral epitope sequence [5,6].

T-cell regulation during pregnancy

Successful pregnancy requires that maternal T cells tolerate paternal alloantigens expressed by the fetus. Failure to do so has been linked to early onset of labor and fetal death [7]. The placental maternal–fetal interface is thus replete with mechanisms to prevent or subdue untoward allogeneic T-cell responses. These include antigen presentation by tolerogenic nonclassical class I molecules, such as HLA-G, tryptophan catabolism by indoleamine 2,3-dioxygenase, inhibition via programmed cell death-1 ligand 1, and expansion of immunosuppressive FoxP3⁺CD4⁺ Tregs, many of which are specific for paternal antigens [8,9]. Although these immunomodulatory mechanisms are

concentrated in the placenta, several also exert influence on systemic cellular immunity during pregnancy. For instance, Tregs with robust immunosuppressive activity expand systemically during pregnancy and may inhibit CD8⁺ T-cell activation, proliferation and cytokine production in the periphery by secreting IL-10 and TGF- β , depleting available IL-2 and secreting extracellular adenosine [10,11]. Furthermore, pregnancy hormones such as progesterone and estrogen have been shown to affect CD8⁺ T-cell activity [12,13]. Whether the systemic immunosuppressive effects of pregnancy further impair the already dysfunctional HCV-specific CD8⁺ T-cell response is not clear. Recognition that HCV viral loads tend to climb during pregnancy and frequently fall sharply after delivery [14,15] supports the hypothesis that HCV-specific T-cell function may be suppressed further during pregnancy and potentially rebound after delivery.

Viral evolution during pregnancy & implications for vertical transmission

Since CD8⁺ T cells are major drivers of the evolution of HCV genomes, we recently attempted to gain insight into the effects of pregnancy on HCV-specific cellular immunity by examining evolution of HCV genomes in serial blood samples of two persistently infected women followed through consecutive pregnancies. Viremia dropped markedly after the first delivery in both women and viruses with amino acid substitutions in one or more HLA class I epitopes emerged. These substitutions prevented recognition by CD8⁺ T-cell lines derived from the women, confirming that they functioned as immune-escape mutations. Surprisingly, several of these effective escape mutations were lost in the second pregnancy, only to appear again after the second delivery, coincident with another sharp fall in viremia. This unusual pattern of amino acid substitution suggested that HCV-specific CD8⁺ T-cell selection pressure was transiently reduced during pregnancy. Using cell culture-adapted HCV, we demonstrated that escape mutations lost in pregnancy significantly impaired *in vitro* production of infectious viruses relative to viruses bearing the ‘wild-type’ epitope sequences. These findings confirmed for the first time that maternal–fetal tolerance mechanisms of pregnancy do indeed impair HCV-specific CD8⁺ T-cell responses. By extension, HCV-specific

CD8⁺ T cells targeting escaped epitopes retain some residual *in vivo* function during chronic infection [14].

This study also yielded insight into the pathogenesis of vertical transmission. Improved viral replication through loss of unfit escape mutations in pregnancy may contribute to higher viral loads that have been linked to increased risk of vertical transmission. One subject in this study had viral loads in excess of 10⁷ IU/ml in both pregnancies and vertical transmission occurred with each. Sequencing of virus from the second child confirmed that he had received the fit wild-type sequence of the epitopes that had lost escape mutations in the mother during pregnancy, suggesting that relaxed CD8⁺ T-cell immune pressure during pregnancy may permit viruses with the highest replicative and infectious capacity to be passed to offspring [14].

Relevance to other viral infections

Pregnancy has been associated with increased disease severity and/or shedding of a variety of RNA and DNA viruses, but for many viral pathogens there is no unique phenotype in pregnancy [16]. HBV and HIV, two vertically transmissible pathogens that share with HCV the capacity to generate CD8⁺ T-cell escape variants, offer instructive illustrations of differing natural histories during pregnancy. For HBV, viral loads dynamics parallel those of pregnant women infected with HCV [17,18]. An increase in HBV antigen titers, as well as DNA replication, is observed during pregnancy, followed by a precipitous decrease in viral load following delivery. In addition, alterations in serum alanine aminotransferase levels indicate liver disease activity is increased postpartum in women chronically infected with HBV [18]. Given the similarities of HCV and HBV viral load patterns, it is tempting to speculate that changes in HBV viral load could relate to relaxed HBV-specific T-cell immunity in pregnancy, and possibly reversal of costly CD8⁺ escape mutations with improved viral replication. Like HCV, HBV viral loads in pregnancy are clinically relevant as they correlate with risk of vertical transmission. Further work is necessary to confirm whether altered CD8⁺ T-cell pressure mediates HBV viral load changes in pregnancy.

Natural history studies of HIV in pregnancy performed prior to the era of standard antiretroviral therapy did not identify significant changes in HIV viral load during or after pregnancy [19],

suggesting that HIV-specific T cells may not be affected by the immunoregulatory changes of pregnancy. Numerous studies of HIV vertical transmission have documented the impact of transmission of CD8⁺ class I epitopes to offspring in HIV [20], but to our knowledge there has been no longitudinal sequencing of maternal viral genomes to determine whether maternal class I escape mutations are lost during pregnancy.

Multiple factors could account for the discrepancies in viral load dynamics of HIV versus HCV and HBV. HCV and HBV are both hepatotropic viruses, suggesting liver-specific immunity might be particularly susceptible to regulation in pregnancy. Alternatively, it could be hypothesized that CD8⁺ T cells specific for HCV and HBV may be more sensitive to the expansion and contraction of Tregs during and after pregnancy than those targeting HIV. Furthermore, even if HIV-specific CD8⁺ T cells are activated postpartum, depletion of CD4⁺ T-cell help in uncontrolled HIV infection may hinder restoration of CD8⁺ T-cell effector function. Given these uncertainties, a comparison of the evolution of HBV and HIV class I epitopes during and after pregnancy could help address the initial question of whether differential regulation of HBV-specific and HIV-specific CD8⁺ T-cell function contributes to their distinct patterns of viremia in pregnancy.

Conclusion

Analysis of HCV evolution during and after pregnancy confirms that HCV-specific CD8⁺ T cells are suppressed during pregnancy and that viruses optimized for replication are vertically transmitted, yielding insight into how viruses dependent on vertical transmission might take advantage of the immunologic niche of pregnancy for perpetuation. Whether similar viral adaptations occur in HBV or other viral infections is not clear, but altered viral dynamics during pregnancy suggest further investigation is warranted. Understanding methods of modulating viral control during pregnancy and parturition may uncover novel strategies for the prevention of vertical transmission and provide new insight into the intricacies of maternal immune regulation.

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