The Human Fetal Immune Response to Hepatitis C Virus Exposure in Utero

Jennifer M. Babik, Deborah Cohan, Alexander Monto, Dennis J. Hartigan-O’Connor, and Joseph M. McCune

1Division of Experimental Medicine and 2Division of Infectious Diseases, Department of Medicine, University of California, San Francisco; 3Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco and San Francisco General Hospital; and 4Division of Gastroenterology, Department of Medicine, University of California, San Francisco and the San Francisco Veterans Affairs Medical Center, San Francisco, California

Background. Although the rate of mother-to-child transmission of hepatitis C virus (HCV) is low, the effect of HCV exposure in utero on the fetal immune system is unknown.

Methods. Umbilical cord blood was obtained from 7 neonates born to HCV-seropositive, HCV RNA-positive women and 8 neonates born to HCV-seronegative women. Cord blood mononuclear cells were analyzed by immunophenotyping and by intracellular cytokine staining after HCV-specific and polyclonal stimulation. Plasma was analyzed for anti-HCV immunoglobulin M (IgM), cytokine/granzyme concentrations, and indoleamine 2,3-dioxygenase (IDO) activity.

Results. HCV-exposed neonates had significantly lower levels of regulatory T cells expressing HLA-DR, lower CD4+ and CD8+ T cell activation, and lower plasma levels of pro-inflammatory markers than did controls. However, CD4+ and CD8+ T cells from HCV-exposed neonates had higher IFN-γ production in response to polyclonal stimulation than did T cells from controls. IDO activity was similar between groups. No HCV-specific T cell responses or anti-HCV IgM were detected in any neonates.

Conclusions. HCV-exposed neonates showed a relative suppression of immune activation and pro-inflammatory markers, which was counterbalanced by an increased production capacity for IFN-γ. These results suggest that HCV encounters the fetal immune system in utero, and alters the balance between suppressive and pro-inflammatory responses.

Hepatitis C virus (HCV) is a major cause of chronic liver disease in both children and adults worldwide [1]. Since the advent of universal screening of blood products, mother-to-child transmission (MTCT) has become the major route of HCV infection in children [2]. It is estimated that 10,000–60,000 newborns worldwide are infected with HCV by MTCT each year [3]. The rate of MTCT from HCV-seropositive, HCV RNA-positive women is 4%–6% and transmission occurs almost exclusively from women who are viremic [2]. Although the timing of transmission is not well defined, it appears that approximately one-third of transmission events occur in utero [4], with the rest occurring peripartum. Risk factors for HCV MTCT include HIV coinfection and intrapartum exposure to maternal blood [2]. Breastfeeding, HCV genotype, and mode of delivery are not associated with MTCT. There are very few studies investigating the biology of HCV MTCT, and the reason for the low rate of transmission remains unexplained. The findings that female sex [5] and the absence of HLA-DR13 in the infant [6] might be risk factors for transmission suggest that the fetal immune system may play a role in protection against and/or facilitation of MTCT.
Fetal exposure to HCV likely occurs more frequently than in utero transmission. Bidirectional trafficking of maternal and fetal cells across the placenta occurs routinely [7, 8], and it is therefore difficult to imagine a scenario whereby viral particles would not also cross the placenta with some regularity. Based on an HCV load of $10^5$–$10^6$ copies/mL and 600 mL/min placental blood flow at term [9], an estimated $10^{13}$–$10^{14}$ HCV virions access the placental bed during gestation, making it highly probable that some particles would cross the placenta even if transfer was inefficient. This led us to ask: if HCV exposure in utero is common, what is the effect of such exposure on the fetal immune system?

The fetal immune environment is skewed toward tolerance and Th2 immune responses to avoid Th1 and pro-inflammatory responses that are toxic to the placental/fetal unit [10–12]. There are multiple mechanisms of maternal-fetal tolerance, including regulatory T cells (Tregs) and the suppressive enzyme indoleamine 2,3-dioxygenase (IDO) [11, 13]. Indeed, recent work from our laboratory has shown that the fetus mounts a Treg response to noninherited maternal antigens on cells that cross into the fetal circulation [7]. We hypothesized that exposure to HCV antigens in utero might elicit a similar suppressive immune response. In this study, we aimed to determine if in utero exposure to HCV altered the fetal immune environment, with particular attention to Tregs, T cell activation and pro-inflammatory markers, IDO activity, and antigen-specific immune responses.

### METHODS

#### Patients and blood samples

Umbilical cord blood (UCB) was obtained from 7 neonates born to HCV-seropositive, HCV RNA-positive women (HCV-exposed group) and 8 neonates born to HCV-seronegative women (control group). All deliveries occurred at San Francisco General Hospital. Basic clinical and demographic data were

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<th>Table 1. Clinical and Demographic Characteristics of Study Groups</th>
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<td>Detectable HCV RNA$^e$</td>
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**NOTE.** Data are shown as the number (%) of subjects, unless otherwise indicated. ALT, alanine aminotransferase; IQR, interquartile range.

$^a$The HCV-exposed group was composed of 7 neonates born to 6 HCV-positive mothers (there was one set of dichorionic-diamniotic twins).

$^b$P values were calculated for the difference between groups using the Mann-Whitney test for comparison of continuous variables and the Fisher exact test or chi-squared test for proportions, as appropriate.

$^c$Birth weight and Apgar score data were available for 6 control subjects.

$^d$ALT testing was performed on umbilical cord blood from 6 controls and 5 HCV-exposed neonates.

$^e$Umbilical cord blood HCV RNA testing was performed on 6 subjects from each group. The detection limit of the assay was 615 IU/mL.

$^f$Maternal pregnancy complications, as determined using standardized diagnostic criteria by the obstetrics service, included preeclampsia, chronic or pregnancy-induced hypertension, and gestational diabetes.

$^g$ALT values were available for 6 of the women in the control group.
collected at the time of delivery (Table 1). All maternal laboratory values were obtained from the medical record and were performed as part of routine clinical care. HCV antibody status was determined by immunoassay (Siemens Healthcare Diagnostics), HCV RNA by the VERSANT HCV RNA 3.0 Assay (Siemens Healthcare Diagnostics), and HCV genotype by sequencing of the 5’ UTR (ARUP Laboratories). All subjects were hepatitis B surface antigen negative. At delivery, UCB was collected from the umbilical vein using sterile cordocentesis to minimize the possibility of maternal blood contamination. Serum was immediately isolated and tested for the level of alanine aminotransferase and HCV RNA (as above). Blood samples from HCV-positive adults were used as positive controls in some assays and were obtained from the Liver Studies Group at the San Francisco Veterans Affairs Medical Center. All samples were collected under protocols approved by the Institutional Review Board at the University of California, San Francisco, that were in accordance with the guidelines of the US Department of Health and Human Services.

Isolation of mononuclear cells
Plasma was separated from whole blood collected in sodium heparin and was stored at −80°C. Cord blood mononuclear cells (CBMC) or peripheral blood mononuclear cells (PBMC) were then isolated by Ficoll density gradient centrifugation (Histopaque-1077, Sigma-Aldrich). For UCB, nucleated red blood cells were removed by a second round of Ficoll density gradient centrifugation [14].

Antibodies
Anti-CD3-Alexa-Fluor-700, anti-CD3-Pacific-Blue, anti-CD4-Pacific-Blue, anti-CD25-PE-Cy7, anti-CD45RA-PE, anti-HLA-DR-APC-Cy7, anti-IL-2-APC, anti-IFNγ-PE-Cy7, anti-TNFα-Alexa-Fluor-700, and purified anti-CD28 and anti-CD49d were purchased from BD Biosciences. Anti-FoxP3-Alexa-Fluor-647 and anti-CD27-APC-Alexa-Fluor-750 were purchased from eBioscience. Anti-CD4-Pacific-Blue, anti-CD8-Pacific-Blue, and anti-CD28-Pacific-Blue were purchased from Invitrogen.

Immunophenotyping
Absolute T cell counts were determined by staining 50 µL of whole UCB in TruCOUNT tubes (BD Biosciences) using anti-CD3-Alexa-Fluor-700, anti-CD4-Pacific-Blue, and anti-CD8-Pacific-Blue according to the manufacturer’s instructions. Phenotyping of fresh CBMC was performed with 5 × 10⁵ cells using anti-CD3-Alexa-Fluor-700, anti-CD4-Pacific-Blue, anti-CD8-Pacific-Blue, anti-CD25-PE-Cy7, anti-CD38-PE-Texas-Red, anti-CD4-Pacific-Blue, anti-CD27-APC-Cy7, and aqua LIVE/DEAD Fixable Dead Cell Stain (Invitrogen). Cells were then fixed, permeabilized, and stained with anti-FoxP3-Alexa-Fluor-647 using the FoxP3 Staining Buffer Set (eBioscience) according to the manufacturer’s instructions. Samples were processed on an LSR-II flow cytometer (BD Biosciences) and data were analyzed using FlowJo software (Treestar, Inc). For each experiment, LSR-II photomultiplier tube voltages were set using rainbow bead target values to minimize variability between sample runs.

Intracellular cytokine staining
Fresh CBMC or PBMC were cultured in RPMI-1640 containing 10% heat-inactivated fetal bovine serum and stimulated with media alone (containing DMSO), overlapping HCV peptides, or phorbol 12-myristate 13-acetate (PMA) (0.01 µg/mL) plus ionomycin (1 µg/mL) (Sigma-Aldrich). The HCV peptides were provided by the NIH Biodefense and Emerging Infections Research Resources Repository and were 12–19-mers, overlapping by 11–12 amino acids and spanning the entire sequence of the genotype 1a H77 strain. The peptides were divided into subgenomic pools (core/E1, E2, NS2/p7, NS3, NS4A/NS4B, NS5A, and NS5B) with an individual peptide concentration of 10 µg/mL and DMSO concentration <0.8%. Cultures were incubated at 37°C for 16 h in the presence of anti-CD28 (2 µg/mL), anti-CD49d (2 µg/mL), and GolgiPlug (BD Biosciences; concentration 1:1000). Cells were then harvested and stained with anti-CD4-PE-Texas-Red, anti-CD8-PE-Cy5.5, anti-CD25-PE-Cy7, anti-CD3-PE-Cy7, and anti-TNFα-Alexa-Fluor-700. Samples were processed and analyzed as described above.

Luminex assay
Plasma samples were analyzed using a custom multiplex assay (LEGENDplex, Biolegend) for granzyme A, granzyme B, interferon (IFN) α, IFN-γ, tumor necrosis factor (TNF) α, interleukin (IL) 2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17A, IL-18, IL-22, and VEGF. The assay was performed as per the manufacturer’s instructions and samples were run in duplicate on a Luminex 100 System instrument (Luminex). Analyte concentrations were determined by interpolation from a standard curve.

IDO activity
Plasma tryptophan and kynurenine concentrations were measured by high-performance liquid chromatography as previously described [15].

Anti-HCV IgM ELISA
Plasma was diluted 1:20 and treated with anti-human immunoglobulin G (IgG) (RF-Absorbent, IBL International) according to the manufacturer’s instructions to remove anti-HCV IgG and rheumatoid factor. Anti-HCV immunoglobulin M (IgM) was then detected using an anti-HCV IgG enzyme-linked
immunosorbent assay (ELISA) kit (CTK Biotech) modified for IgM detection by using a horseradish peroxidase-conjugated goat anti-human IgM antibody (Bethyl Labs; dilution 1:9000). All samples were run in triplicate and read at an optical density (OD) of 450 nm on a SpectraMax M2 microplate reader (Molecular Devices). An OD was considered positive if it was greater than the mean $\pm$ 2 SD of all seronegative samples. Plasma from an adult HCV-positive subject with anti-HCV IgM detected in preliminary testing was used as a positive control.

Statistical analysis
The Mann-Whitney test was used for comparison of continuous variables and the Fisher exact test or $\chi^2$ test was used for proportions, as appropriate. Correlation was performed by Spearman’s correlation. Univariate analysis of baseline characteristics and immunologic results was performed using linear regression. Two-sided $P$ values were calculated for all test statistics and $P < .05$ was considered significant. Statistical analyses were performed using GraphPad Prism software, version 5.01 (GraphPad).

RESULTS

Study group characteristics
There were no significant differences in the clinical or demographic characteristics between study groups (Table 1). The HCV-exposed group had a trend toward a lower gestational...
age and lower birth weight, but both groups were delivered at a median gestational age >37 weeks (full term), and all birth weights were appropriate for gestational age. There was one set of dichorionic-diamniotic (fraternal) twins in the HCV-exposed group. One of 6 HCV-exposed neonates tested had a detectable cord blood HCV RNA level, suggesting in utero infection [2]. This neonate was born to an HIV/HCV-coinfected woman.

HCV-exposed neonates had similar numbers of Tregs but lower levels of HLA-DR^+ Tregs than controls
We first sought to determine the levels of Tregs in each group by immunophenotyping of CBMC. We found that HCV-exposed neonates and controls had a similar percentage and absolute number of Tregs, defined as CD25^+FoxP3^+CD4^+ T cells (Figure 1A, 1B). In addition, the overall FoxP3 MFI on Tregs was similar between groups. However, HCV-exposed neonates had

Figure 2. HCV-exposed neonates had lower levels of T cell activation than controls. A, Representative flow plots showing the gating strategy for the activation markers CD38 and HLA-DR on CD4^+ and CD8^+ T cells. B, Scatter plots showing the absolute number of HLA-DR^+, CD38^+, and HLA-DR^+CD38^+ CD4^+ T cells (top panels) and CD8^+ T cells (bottom panels). Horizontal lines represent the median. To ensure that the apparent outlier in the HLA-DR^+ and HLA-DR^+CD38^+ control data did not exert undue influence on our results, a nonparametric test (Mann-Whitney) was used to calculate P values. The HCV-exposed neonate with a positive cord blood HCV RNA level is indicated by an open triangle (Δ). C, Spearman correlations of the percentage of HLA-DR^+ Tregs with the percentages of HLA-DR^+CD4^+ and HLA-DR^+CD8^+ T cells. HCV-exposed neonates are indicated by open shapes and control neonates by closed shapes.
Figure 3. HCV-exposed neonates produced more IFN-γ in response to polyclonal stimulation than controls. A, Representative flow plots showing IFN-γ, TNF-α, and IL-2 production from CD4^+ and CD8^+ T cells after polyclonal stimulation and intracellular staining. 2.5 × 10^5 fresh cord blood mononuclear cells were stimulated with phorbol 12-myristate 13-acetate (PMA) plus ionomycin for 16 h in the presence of purified anti-CD28, anti-CD49d, and GolgiPlug. All negative control stimulations (media containing DMSO only) had background levels less than .25%. B, Scatter plots showing the aggregate exposure to Hepatitis C Virus in Utero • JID 2011:203 (15 January) • 201
a 3.6-fold lower percentage of HLA-DR\(^+\) Tregs than did controls (Figure 1B). HLA-DR\(^+\) Tregs are a distinct subset of Tregs that have been shown to have higher FoxP3 levels and to induce a more rapid suppression of effector T cells than do HLA-DR\(^-\) Tregs [16]. Although our CBMC numbers limited our ability to perform functional Treg assays, we found that HLA-DR\(^+\) Tregs from both HCV-exposed and control neonates had a 2- to 3-fold higher FoxP3 MFI than did HLA-DR\(^-\) Tregs (Figure 1C).

**HCV-exposed neonates had lower levels of T cell activation but a similar maturation profile as controls**

Based on the finding that HCV-exposed neonates had lower levels of HLA-DR\(^+\) Tregs, we next sought to determine whether the study groups also had different levels of T cell activation (Figure 2A). We found that HCV-exposed neonates had significantly lower numbers of HLA-DR\(^+\)CD4\(^+\), HLA-DR\(^+\)CD8\(^+\), CD38\(^+\)CD8\(^+\), and CD38\(^+\)HLA-DR\(^+\)CD8\(^+\) T cells compared with controls (Figure 2B). Results for the percentages of activated CD4\(^+\) and CD8\(^+\) T cells showed similar results (data not shown). The percentage of HLA-DR\(^+\) Tregs was strongly correlated with the percentage of HLA-DR\(^+\)CD4\(^+\) and HLA-DR\(^+\)CD8\(^+\) T cells (Figure 2C).

To determine whether these differences in T cell activation could be explained by differences in maturation profile between groups, we categorized T cell subsets using the maturation markers CD45RA and CD27 [17]. There were no significant differences between groups in the proportion of naive, central memory, or effector memory CD4\(^+\) or CD8\(^+\) T cell subsets (data not shown).

**HCV-exposed neonates produced more IFN-\(\gamma\) after polyclonal stimulation than did controls**

Given the phenotypic differences in T cells from HCV-exposed and control neonates, we looked for differences in T cell data for cytokine production from CD4\(^+\) T cells (top panels) and CD8\(^+\) T cells (bottom panels) after stimulation with PMA and ionomycin. Horizontal lines represent the median. The HCV-exposed neonate with a positive cord blood HCV RNA level is indicated by an open triangle (\(\Delta\)).

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**Figure 4.** HCV-exposed neonates had lower granzyme and pro-inflammatory cytokine levels than controls. Scatter plots showing the levels of granzyme A, granzyme B, IL-12, IL-17A, and IL-18 determined by multiplex assay, which was performed on plasma from 7 controls and 6 HCV-exposed neonates. Horizontal lines represent the median, and \(P\) values were calculated using the Mann-Whitney test. The HCV-exposed neonate with a positive cord blood HCV RNA level is indicated by an open triangle (\(\Delta\)).

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function between groups. We measured the capacity of CD4$^{+}$ and CD8$^{+}$ T cells to make IL-2, TNF-α, and IFN-γ in response to polyclonal stimulation with PMA and ionomycin (Figure 3A). HCV-exposed neonates had a 3.5-fold higher percentage of CD4$^{+}$ and CD8$^{+}$ T cells making IFN-γ compared with controls, but there was no difference in the production of IL-2 or TNF-α between groups (Figure 3B). A similar pattern of results was seen for the absolute numbers of CD4$^{+}$ and CD8$^{+}$ T cells making cytokines (data not shown). The majority of CD4$^{+}$ and CD8$^{+}$ T cells making IFN-γ were naive T cells, but among IFN-γ$^{+}$CD4$^{+}$ T cells, there was a 12-fold higher proportion of terminally differentiated effector memory (TEMRA) cells in the HCV-exposed group compared with controls (Figure 3C). There were no differences between groups in the maturation profile of IFN-γ$^{+}$CD8$^{+}$ T cells.

**HCV-exposed neonates had lower levels of pro-inflammatory cytokines and granzymes than controls, but similar levels of IDO activity**

To further characterize any differences in pro-inflammatory markers between groups, we performed a multiplex analysis of UCB plasma. HCV-exposed neonates had significantly lower levels of granzyme A, IL-12, and IL-17A and a trend toward lower levels of granzyme B and IL-18 (Figure 4). There were no differences between groups in the levels of IL-4, IL-6, IL-8, IL-10, IL-22, and VEGF (data not shown). IFN-α, IFN-γ, IL-2, IL-4, IL-13, and TNF-α were undetectable by this assay.

We next sought to determine if the immunosuppressive enzyme IDO might explain the differences in pro-inflammatory markers and T cell activation between groups. IDO is the rate-limiting enzyme in the breakdown of tryptophan through the kynurenine pathway, and its activity can be measured by determining the plasma concentrations of these analytes [15]. We found no differences in the level of tryptophan, kynurenine, or the kynurenine/tryptophan ratio between groups (data not shown).

**HCV-specific immune responses were not detectable in HCV-exposed neonates**

To determine the antigen-specific T cell functionality in HCV-exposed neonates, we stimulated CBMC with overlapping HCV peptide pools and measured T cell production of IL-2, TNF-α, and IFN-γ. This assay was tested in 29 HCV-positive adults, and a CD4$^{+}$ or CD8$^{+}$ T cell cytokine response was detected in more than two-thirds of subjects, independent of HCV RNA level or genotype (Figure 5; data not shown). However, none of the HCV-exposed neonates had a detectable cytokine response (Figure 5).

To determine if HCV-exposed neonates mounted an antibody response against HCV, we measured the level of anti-HCV IgM in UCB plasma by ELISA. Although only 30%–80% sensitive for HCV infection [18–20], anti-HCV IgM is very specific to the fetal compartment because IgM, unlike IgG, does not cross the placenta [21, 22]. However, no anti-HCV IgM was detected in any of the HCV-exposed neonates (data not shown).

**Baseline characteristics and immunologic results**

There was no significant association by univariate analysis of gestational age, birth weight, or maternal HCV RNA level with any of the immunologic parameters that differed between groups (data not shown). The HCV-exposed neonate who was

![Figure 5](https://academic.oup.com/jid/article-abstract/203/2/196/911915)
HCV RNA-positive had the highest number of overall Tregs, the highest FoxP3 MFI, the lowest fraction of HLA-DR⁺ Tregs, and the lowest level of IL-18 (Figures 1B and 4). Exclusion of these data points from their respective analyses did not alter our findings (data not shown), and results of other assays for this neonate were at or near the median.

DISCUSSION

Our results show that HCV-exposed neonates had a relative suppression of T cell activation and pro-inflammatory markers compared with controls, which was offset by a higher production capacity for IFN-γ. This indicates a balance between pro- and anti-inflammatory responses in the HCV-exposed neonate. To our knowledge, this is the first description of the immunologic characteristics of UCB from HCV-exposed neonates.

Taken together, these results suggest that HCV contacts the fetal immune system in utero, likely via transplacental passage. There are several potential sites in the placenta for the passage of free or cell-associated virus [23], but it is also possible that HCV may directly infect the placenta. Many of the putative HCV receptors and attachment factors have been detected in the placenta, including claudin-1, occludin, SR-B1, LDLr, and DC-SIGN [24–26]. Alternatively, it is possible that HCV proteins cross the placenta as soluble antigens rather than as whole virions. In particular, the HCV core protein can be secreted in soluble form and has known immunomodulatory effects including the suppression of T cell proliferation and activation [27, 28]. An attractive hypothesis is that the HCV core protein may be playing a role in the alteration of fetal immunity in utero.

While it seems likely that HCV crosses into the fetal circulation and contacts the fetal immune system, antigen-specific immune responses were not detected in HCV-exposed neonates. One possible explanation is that neonatal responses were below the assay limit of detection despite the excellent sensitivity of these assays for adult samples. Second, it is possible that HCV responses were being actively suppressed or resulted in Th2 cytokine production, and experiments to test these hypotheses are ongoing. Third, it is possible that HCV might not contact the fetal immune system directly. For example, the increased IFN-γ production seen in HCV-exposed neonates could be a result of maternal pro-inflammatory cytokines [29] or anti-idiotypic antibodies that mimic exposure to HCV antigens [30, 31]. These possibilities seem less likely, but we were unable to exclude them entirely because we did not have paired maternal blood samples. It is also possible that maternal chronic liver disease itself, irrespective of HCV, might have indirectly affected the immune system of the HCV-exposed neonates, perhaps as a result of maternal cytokine dysregulation [32]. We did not have any women in the control group with chronic liver disease in order to test this hypothesis because liver disorders in pregnancy are rare [33], but no woman in either group had cirrhosis and there were no differences in maternal ALT values between groups. Last, it is possible that the innate immune system or unconventional T cell responses might play a larger role than classic adaptive immune responses in protecting the fetus from in utero infection [34–38].

What mechanism underlies the altered immune balance seen in HCV-exposed neonates? The levels of Tregs, IL-10, and IDO activity were not different between groups. HCV-exposed neonates did have lower levels of HLA-DR⁺ Tregs, a subset of Tregs that are reported to be more highly suppressive [16]. However, because the level of HLA-DR⁺ Tregs was highly correlated with the level of CD4⁺ and CD8⁺ T cell activation, the presence of HLA-DR⁺ Tregs might in fact be a surrogate marker of immune activation in this setting. Another possible mechanism for the altered immune balance in HCV-exposed neonates is the immunomodulatory HCV core protein, as discussed above. Finally, it is possible, although unlikely, that the frequency of unmeasured maternal coinfections was higher in the control group, thereby resulting in greater in utero antigen exposure and T cell activation.

Although most of the pro-inflammatory markers and immune activation indices were lower in HCV-exposed neonates, IFN-γ production capacity was higher. This balance between pro- and anti-inflammatory mechanisms in response to infectious disease exposure in utero has been described elsewhere [39–41]. On the one hand, low levels of T cell activation may be beneficial in promoting T cell function for the clearance of HCV [42]. On the other hand, IFN-γ produced in response to in utero exposure might play a role in protecting infants from infection, as in the case of HIV [29, 40]. Therefore, the balance of pro- and anti-inflammatory mechanisms might serve to protect against in utero HCV infection. Indeed, 5 of 6 neonates tested did not show evidence of in utero infection. The mechanism behind the increased IFN-γ production in HCV-exposed neonates remains to be determined, but one possibility is via an epigenetic process that reverses the neonatal hypermethylation of the IFN-γ promoter [43]. Given our finding that HCV-exposed neonates had a higher proportion of TEMRA cells among IFN-γ⁺CD4⁺ T cells, it is also possible that HCV exposure alters the maturation pathway of memory T cells.

The clinical effect of this altered fetal immune balance in HCV-exposed neonates remains to be determined. For example, will the increased IFN-γ production decrease the incidence of allergy or augment the clinical response to vaccination and infection? The maintenance of a Th2 skew and poor IFN-γ production after birth have been associated with the development of asthma and weaker responses to vaccination [44, 45]. Conversely, the presence of a Th1-biased immune response in the setting of congenital Trypanosoma cruzi infection has been shown to augment the IFN-γ response to neonatal vaccination.
Longitudinal studies that follow the clinical outcomes of HCV-exposed infants are needed. This study has several limitations. Our small sample size may have limited our ability to find subtle differences in immune parameters with considerable biological variation, such as Tregs. However, our results showed a consistent pattern across multiple assays and for both absolute numbers and percentages, thereby lending increased credibility to these findings. That said, our conclusions are still preliminary and should be confirmed in a larger study of HCV-exposed neonates. Another potential limitation is that we could not compare maternal immune responses between groups, which could have provided insight into differences seen in the neonates. This is an important component to include in future studies of HCV-exposed neonates. Finally, it is possible that the inclusion of one neonate born to an HIV/HCV-co-infected mother could have influenced the results of our HCV-exposed group. However, in the assays in which this neonate’s results were not near the group median, exclusion from the analysis did not alter our results.

Our findings indicate that neonates exposed to HCV in utero have an altered immune reactivity manifest as a relative suppression of immune activation and pro-inflammatory markers that was counterbalanced by an increased production capacity for IFN-γ. These results suggest that HCV encounters the fetal immune system in utero and alters the balance between suppressive and pro-inflammatory responses. More research on the mechanisms of HCV transmission across the placenta and its effects on the fetal immune system are needed. These results have important implications for our understanding of the fetal immune response to antigens encountered in utero and may contribute to the development of interventions aimed at interrupting the MTCT of infectious agents.

Acknowledgments

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References