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HLA DR13 and HCV Vertical Infection

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ABSTRACT

Risk factors affecting vertical hepatitis C virus (HCV) transmission are not completely known, if we exclude maternal HIV coinfection. We hypothesized that immunogenetic factors related to maternal or neonatal HLA profiles may affect HCV vertical transmission. HLA typing (microcytotoxicity assay on blood samples) was performed in 18 infants affected by vertically transmitted HCV infection and in 17 serum-reverted infants. (Serum-reversion is defined as antibody negative by 1 year of age and persistently HCV-RNA negative.) Moreover, HLA typing was performed in 20 mothers. Logistic regression analysis showed a significant negative association between children's HLA-DR13 antigens and risk of HCV vertical transmission ($p < 0.01$). This association persisted in a model including the maternal HIV status: HLA DR13 and maternal HIV coinfection showed a separate, opposite effect on vertical HCV infection ($p < 0.01$ and $p < 0.001$, respectively). The relative risk estimate for the ratio of not-infected to infected children in the presence of DR13 was 8.4 (95% confidence bounds, 1.1–60.8). Breastfeeding did not affect the risk of vertical HCV transmission. Maternal HLA profile did not relate to vertical infection. The present study reveals a significant association between HLA-DR13 and the likelihood of seroreversion in infants born to HCV-infected mothers. The findings of the present study could help in better understanding the pathogenesis of vertical HCV infection and in better identifying the cases at higher risk, which would be useful for the development of prevention strategies. It is possible that DR13 modulates the immune response to viruses, enhancing their clearance and, thus, in the case of HCV, exerting a protective role against the development of vertical infection. **(*Pediatr Res* 51: 746–749, 2002)**

Abbreviations

HCV, hepatitis C virus
RT-PCR, reverse transcriptase-PCR

Infants born to HCV-infected mothers are at risk of vertical infection. The transmission rate ranges from almost 3.5% to 15%, the higher risk being associated with maternal HIV coinfection (1). No association has been found between breastfeeding and HCV transmission; also type of delivery has no effect on the risk of infection although definitive conclusions can be provided only by larger data sets (2). Other factors have been studied, such as maternal viral load, but definitive results are not available at the moment. The role of the immune response related to the HLA profile has been extensively studied in HCV-infected adults, especially concerning the severity of liver disease. In this respect it has been shown that specific HLA class II antigens (DR4, DR11, DR12, DR13, DR16) are able to present some specific HCV epitopes stimulating an acute T-cell response with consequent viral elimination (3). Also some HLA class I antigens have been shown to be able to enhance the immune response to HCV (4). No studies are available on the possible correlation between HLA genotype and risk of vertical HCV infection.

The present study was conducted to investigate the possible role of maternal and children's HLA genotype on the risk of vertical HCV infection.

METHODS

Subjects. HLA typing was performed in 35 children and in their 33 mothers (two women had two children, not twins). The children are part of a group of 350 infants born to HCV-positive mothers followed since 1990 in our unit. Rate of vertical HCV transmission in the total population is 15.6% and 2.4% in infants born to HIV- and non-HIV–coinfected mothers, respectively ($p = 0.007$; data transmitted to the European Pediatric HCV Infection Network). Type of lactation and type of delivery did not affect the risk.

In a subgroup of 28 of 35 children, the selection criteria for the HLA determination were the following: birth to HCV viremic mothers and requirement of a blood sample in the period between January and May 2000. Maternal HCV viremia was defined on the basis of a positive HCV-PCR. Seven HCV vertically infected children were also recalled to perform HLA

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testing to obtain numerically comparable groups of infected and not-infected children. The selection was based on the age of the children: we recalled the older infected children with the exception of two whose parents did not give consent for the study.

Eighteen of 35 children were HCV vertically infected, and 17 children were serum-reverted (antibody negative by 1 y of age and persistently HCV-RNA negative). Children's infection was defined on the basis of persisting anti-HCV antibodies for at least 18 mo and positive RNA-PCR in two consecutive determinations. In two cases HCV-RNA was detected immediately after birth, thus suggesting, as reported by other authors (5), *in utero* transmission of HCV. In the remaining 16 cases HCV-RNA was detected between the third and sixth months of life.

Maternal HIV status and perinatal data in the two groups of infected and serum-reverted infants are reported in Table 1. Among the study infants there was no HIV infection. Mono- or biparental consent was asked in all cases to obtain blood samples for HLA determination. Moreover, HLA was assayed in 20 of the 33 mothers. The 20 mothers were not related. In 13 cases the absence of the mother during the examination prevented the collection of blood samples. All study subjects were white.

Measurements. Serum HCV-RNA was determined by nested RT-PCR by Beckman Analytical (Milan, Italy) that uses conserved primers localized in the 5' noncoding region of the viral genome, using RNA extracted from 100 μL of serum. HLA typing was performed on blood samples using a microtoxicity assay (6).

Data analysis. Data were collected in a Microsoft Excel (Microsoft Corp., Redmond, WA, U.S.A.) database and were analyzed using the statistical package SPSS v. 5.0 (SPSS Inc., Chicago, IL, U.S.A.). Logistic regression analysis, stepwise method with likelihood ratio statistics, was used to study the possible correlation between HLA pattern and risk of vertical infection. The HLA class I antigens (A, B, C) and the HLA class II antigens (DR and DQ) were separately considered in five statistical models. Maternal HIV coinfection and type of lactation were also introduced in the statistical models.

RESULTS

Maternal and children's relative frequencies of the different HLA classes are reported in Table 2. Logistic analysis showed no significant association between maternal HLA and risk of vertical HCV infection. When children's HLA was analyzed, only HLA DR13 antigen showed a significant negative association with the risk of acquiring vertical HCV infection ($p = 0.0075$). This association persisted in a model comprehending the maternal HIV status; HLA DR13 and maternal HIV coinfection showed a separate, opposite effect on vertical HCV infection ($p = 0.0028$ and $p = 0.0006$, respectively). HLA DR13 was present in seven of 17 (41.2%) serum-reverted infants *versus* one of 18 (5.6%) infected infants. The relative risk estimate for the ratio not-infected to infected children in the presence of DR13 was 7.4 (95% confidence bounds, 1.0–54.1). Neonatal type of lactation did not affect the risk of vertical HCV transmission.

Table 1. *Maternal HCV-HIV coinfection, type of lactation, and type of delivery in the two groups of vertically HCV-infected and seroreverted children*

	Maternal HIV coinfection (*n*)	Breast milk (*n*)	Cesarean section (*n*)
Infected children	7/18	6/18	8/18
Seroreverted children	1/17	4/17	6/17

DISCUSSION

Specific predictors of vertical HCV infection have not yet been identified. Balance between risk factors and host defenses could affect the risk. Possible risk factors such as viral genotype, type of delivery, and type of feeding have shown little or no importance; contrasting data exist regarding viral load, and a precise cutoff level has not yet been indicated (2, 7, 8). Some authors suggested that the role of the immune defensive system could better explain the pathogenesis of HCV infection (9). HLA profile can affect immune response.

In the present study we found a clear tendency toward a lower vertical HCV infection in children presenting the HLA DR13 antigen. This finding suggests the principal role of immunologic host protective mechanisms instead of viral characteristics in the pathogenesis of vertical HCV infection. Moreover, the absence of a specific immune response in noninfected newborns suggests the potential role of aspecific instead of specific immunologic response.

The clearance of HCV soon after birth, in some HCV-RNA–positive newborns born to infected mothers, without the development of specific antibody response has been reported (10). It may be supposed that the rate of HCV maternal-fetal transmission during gestation or delivery is higher than HCV neonatal vertical infection. Therefore, some fetuses or newborns may be more able than others to clear the virus by aspecific defensive mechanisms that do not leave an immune memory. This suggests the important role of the early aspecific defensive immune systems to determine abortive infection. Cells involved in the first aspecific immune phase are generally natural killer cells and mononuclear phagocytes. The extracellular maternal HCV is probably the principal cause of vertical HCV transmission. Extracellular viruses are principally cleared by neutralizing antibodies and by monocytes/macrophages that ingest and clear the virus (11).

HLA class II antigens are involved in the CD4 activation, leading to lymphokine production and subsequent activation of previously quiescent mononuclear phagocytes. Interferon-γ is the most important cytokine for mononuclear phagocyte activation and interferon-γ is a potent inducer of class II HLA surface expression (11). Among HLA class II antigens, DR13 antigen has been associated with resistance to malaria and hepatitis B virus (12) and with longevity (13) in previous studies. Moreover, an increased frequency of DR13 alleles in HIV-negative or seroreverted infants with respect to infected infants has been reported (14), as well as a slower progression of HIV infection in white people presenting HLA DR13 (15).

On the basis of the literature and our data, it may be supposed that HLA DR13 is particularly effective in enhancing

Table 2. *Relative frequencies of the different HLA classes in both infected and seroreverted children (*n = *35) and in 20 of their mothers*

	Mothers		Infants	
	HCV vertically infected children ($n = 8$)	Serum-reverted children ($n = 12$)	HCV vertically infected ($n = 18$)	Serum-reverted ($n = 17$)
A1	12.5	8.3	11.1	11.8
A2	50.0	49.8	38.9	23.5
A3	37.5	24.9	38.9	17.6
A11	12.5	16.6	11.1	17.6
A23(9)	0	0	5.6	5.9
A24(9)	12.5	41.5	33.3	35.3
A26(10)	12.5	0	5.6	0
A28	12.5	0	5.6	11.8
A29	25.0	0	16.7	5.9
A30	0	8.3	5.6	23.5
A32	0	0	0	11.8
A33	0	8.3	5.6	5.9
A69(28)	0	0	5.6	0
B7	12.5	0	16.7	17.6
B8	0	8.3	11.1	5.9
B13	0	16.6	5.6	17.6
B14	0	24.9	0	11.8
B18	12.5	8.3	16.7	11.8
B35	25.0	41.5	27.8	35.3
B38(16)	12.5	16.6	5.6	11.8
B44(12)	25.0	16.6	33.3	17.6
B47	0	0	0	5.9
B49(2)	25.0	0	5.6	0
B51(5)	25.0	24.9	16.7	17.6
B53	12.5	8.3	0	0
B57(17)	25.0	0	11.1	11.8
B58(17)	0	0	0	5.9
B62(15)	12.5	0	11.1	11.8
B63(15)	0	0	0	5.9
B65(14)	0	0	5.6	5.9
Bw4	75.0	66.4	72.2	76.5
Bw6	50.0	74.7	61.1	76.5
Cw3	25.0	0	11.1	11.8
Cw4	25.0	41.5	27.8	29.4
Cw5	12.5	8.3	0	11.8
Cw6	25.0	16.6	27.8	41.2
Cw7	12.5	16.6	33.3	35.3
DR1	25.0	24.9	27.8	23.5
DR3	0	8.3	16.7	5.9
DR4	25.0	16.6	22.2	5.9
DR7	25.0	24.9	44.4	41.2
DR8	0	8.3	0	11.8
DR11(5)	25.0	33.2	33.3	41.2
DR13(6)	12.5	33.2	5.6	41.2
DR14(6)	0	8.3	0	11.8
DR15(29)	0	8.3	22.2	11.8
DR52	37.5	74.7	44.4	70.6
DR53	50.0	41.5	44.4	29.4
DR51	0	8.3	16.7	11.8
DQ1	25.0	58.1	44.4	58.8
DQ2	25.0	33.2	44.4	29.4
DQ3	37.5	8.3	38.9	23.5
DQ4	0	8.3	0	11.8
DQ7(3)	25.0	33.2	16.7	35.3
DQ8(3)	12.5	0	0	0
DQ9(3)	0	0	0	5.9

the immune response to various diseases and, with regard to HCV infection, especially the aspecific immune response. In the present study we could not demonstrate a clear influence of maternal HLA profile on vertical HCV transmission. Unfortunately, the absence of maternal viral load determination in this study does not permit us to verify whether there was an association between maternal HLA profile and viral load.

One limitation of this study is the technique used in determining HLA antigens. Using a molecular technique, not available at the moment of the study, in our laboratories, HLA

DR13 could have been subcategorized into almost 44 different antigens. Studies tracking the cases at this level could provide more definitive evidence and could determine whether there are the same or different splits of DR13 in the children. Moreover, owing to the small size of the sample, it is possible that other HLA antigens could also affect the risk of vertical transmission of HCV, so a larger study is needed.

The findings of the present study could help in better understanding the pathogenesis of vertical HCV infection and in better identifying the cases at higher risk, which would be useful for development of prevention strategies.

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