Antiviral Research 157 (2018) 27–37

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

TLR7 polymorphism, sex and chronic HBV infection influence plasmacytoid DC maturation by TLR7 ligands

Sonja I. Buschow[a], Paula J. Biesta[a], Zwier M.A. Groothuismink[a], Nicole S. Erler[b], Thomas Vanwolleghem[a,c], Erwin Ho[c], Isabel Najera[d], Malika Ait-Goughoulte[d], Robert J. de Knegt[a], Andre Boonstra[a], Andrea M. Woltman[a,*]

[a] Department of Gastroenterology and Hepatology, Erasmus MC University Medical Center Rotterdam, The Netherlands
[b] Department of Biostatistics, Erasmus MC University Medical Center Rotterdam, The Netherlands
[c] Laboratory of Experimental Medicine and Pediatrics, Faculty of Medicine and Health Sciences, University of Antwerp and Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerp, Belgium
[d] Roche Pharma Research & Early Development (pRED), Roche Innovation Center Basel, Switzerland

ARTICLE INFO

Keywords:
Plasmacytoid dendritic cell
TLR7
rs179008
Sex
Hepatitis B virus
Immunotherapy

ABSTRACT

TLR7 agonists are of high interest for the treatment of cancer, auto-immunity and chronic viral infections. They are known to activate plasmacytoid dendritic cells (pDCs) to produce high amounts of Type I Interferon (IFN) and to facilitate T and B cell responses, the latter with the help of maturation markers such as CD40, CD80 and CD86. The TLR7 single nucleotide polymorphism (SNP) rs179008 (GLn11Leu), sex and chronic viral infection have all been reported to influence pDC IFN production. It is unknown, however, whether these factors also influence pDC phenotypic maturation and thereby IFN-independent pDC functions. Furthermore, it is unclear whether SNP rs179008 influences HBV susceptibility and/or clearance.

Here we investigated whether the SNP rs179008, sex and HBV infection affected phenotypic maturation of pDCs from 38 healthy individuals and 28 chronic HBV patients. In addition, we assessed SNP prevalence in a large cohort of healthy individuals (n = 231) and chronic HBV patients (n = 1054).

Consistent with previous reports, the rs179008 variant allele was largely absent in Asians and more prevalent in Caucasians. Among Caucasians, the SNP was equally prevalent in healthy and chronically infected males. The SNP was, however, significantly more prevalent in healthy females than in those with chronic HBV infection (42 versus 28%), suggesting that in females it may offer protection from chronic infection. *Ex vivo* experiments demonstrated that induction of the co-stimulatory molecules CD40 and CD86 by TLR7 ligands, but not TLR9 ligands, was augmented in pDCs from healthy SNP-carrying females. Furthermore, CD80 and CD86 upregulation was more pronounced in females independent of the SNP. Lastly, our data suggested that chronic HBV infection impairs pDC maturation. These findings provide insight into factors determining TLR7 responses, which is important for further clinical development of TLR7-based therapies.

1. Introduction

Toll Like Receptor 7 (TLR7) is of interest as a therapeutic target for chronic viral infections, including those with Human Immunodeficiency virus (HIV), Hepatitis B virus (HBV) and Hepatitis C virus (HCV), and also for other non-infectious diseases such as cancer, asthma and autoimmunity (Savva and Roger, 2013; Funk et al., 2014; Boonstra et al., 2011). The TLR7 gene is located on the X-chromosome. The receptor is expressed intracellularly on plasmacytoid dendritic cells (pDCs) and recognizes viral single stranded RNA (Gibson et al., 2002). Besides TLR7, human pDCs also express autosomal TLR9 recognizing unmethylated CpG DNA (Krug et al., 2001). Upon TLR ligation, pDCs secrete high amounts of Interferon α (IFNα) inducing a potent anti-viral response in neighboring cells (Funk et al., 2014). In addition, TLR ligation induces pDC phenotypic maturation, characterized by upregulation of co-stimulatory molecules such as CD40, CD80 and CD86 and by the secretion of pro-inflammatory cytokines (Gibson et al., 2002; Krug et al., 2001). Via these and other receptors and cytokines, pDCs communicate with other immune cells and exert also IFN-independent immune functions, such as antigen presentation to T cells or facilitating B cell differentiation (Mathan et al., 2013). Both the induction of IFNα and adaptive immunity by pDCs are considered important for TRL7

* Corresponding author.
E-mail address: A.Woltman@erasmusmc.nl (A.M. Woltman).

https://doi.org/10.1016/j.antiviral.2018.06.015
Received 15 January 2018; Received in revised form 21 June 2018; Accepted 25 June 2018
Available online 28 June 2018

agonists to combat viral infection (Funk et al., 2014; Swiecki et al., 2010). To optimize and personalize treatment and to select patients that benefit most from TLR7 agonist treatment, it is important to understand how genetic and environmental factors influence the response of pDCs to TLR7 agonists.

Previously, the TLR7 SNP rs179008 (A/T; Gln/Leu) has been shown to alter TLR7 function. PBMCs from males carrying the variant T allele secreted less IFNα upon TLR7 ligation than those from males with the more common A (i.e. WT) allele (Oh et al., 2009). Furthermore, in HIV, the variant allele associated with higher infection rates, viral load and disease progression (Oh et al., 2009; Said et al., 2014). In HCV, the variant was more prevalent among chronic patients as compared to healthy controls and related to a poor IFNα treatment response (Schott et al., 2007a; Askar et al., 2010). For HBV, the prevalence of the variant allele among chronic patients has not yet been explored.

pDC function is also affected by sex: male pDCs, as compared to female pDCs, produce less IFNα upon TLR7 but not TLR9 ligation (Berghöfer et al., 2006; Meier et al., 2009). This difference is believed to derive from expression differences of X-chromosomal genes downstream of TLR7, combined with hormonal effects (Seillet et al., 2012, 2013; Griesbeck et al., 2015). In addition, chronic HBV (CHB) infection itself can also impair pDC function; HBV impairs IFNα secretion and other pDC functions in response to TLR9 ligation (Woltman et al., 2011; Martinet et al., 2012a, 2012b). Whether rs179008, sex or CHB affect pDC phenotypic maturation in response to TLR7 ligands is not known.

Here we performed an elaborate screen of healthy individuals and CHB patients for their rs179008 genotype. In addition, on PBMCs from healthy and CHB patients, we assessed how this SNP, sex and CHB influence TLR-ligand induced pDC phenotypic maturation. Our results indicate that for CHB the rs179008 variant is not a risk factor and is even underrepresented in Caucasian female patients. On pDCs, both CD40 and CD86 were more induced on variant carrying females. Upregulation of co-stimulatory molecules on pDCs in response to TLR7, but not TLR9, was significantly higher in females than in males. Lastly, our results suggest that in CHB patients TLR7 ligand-induced pDC maturation may be suppressed. Together, these data demonstrate that rs179008 genotype, sex and chronic viral infection influence the response to TLR7 agonist therapy or pathogens that act via TLR7.

2. Methods

2.1. Sample collection and rs179008 genotyping

Blood samples were collected in EDTA or SST tubes. Patients were either CHB patients attending the outpatient clinic of Erasmus MC (Rotterdam, The Netherlands), participants in the 99-01 or PARC study (Janssen et al., 2005; Rijckborst et al., 2010), or members of the Chinese community participating in viral hepatitis outreach screening events (Antwerp, Belgium). The study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. Written informed consent was obtained from all individuals. The ethical review board of Erasmus MC (Rotterdam, the Netherlands) and Antwerp University Hospital (Belgium) approved use of archived serum for this study.

Competitive allele-specific PCR assays (KASP, LGC genomics, Huddleston, UK) were employed for the detection of the reference SNP TLR7 rs179008. Whole blood or serum samples stored at −20 °C or −80 °C were used for DNA extraction and genotyping procedures, which were carried out centrally at LGC genomics as before (Brouwer et al., 2014; Maan et al., 2015). Purified genomic DNA was used for genotyping. The genotype sequence was derived from NCBI.

2.2. Flow cytometry

Peripheral blood mononuclear cells (PBMC) were isolated from venous blood by Ficoll density centrifugation (Ficoll-Paque™ plus, Amersham) and frozen at −150 °C. PBMC were thawed and washed with RPMI Glutamax medium (Life Technologies) with 10% fetal calf serum (FCS; Sigma). 1,000,000 PBMC were stimulated in a 96-well plate in 250 µl RPMI glutamax medium containing 10% FCS, 100 U/ml penicillin/streptavidin (Gibco) and 10 mM Hepes (Lonza) using various stimuli. The cells were incubated with 20 ng/ml IL-3 (Miltenyi) alone or in combination with either 10 µg/ml CpG-A (Invivogen), 0.4 mM Loxoribin (Invivogen) or 0.5 µg/ml R848 (Invivogen). For all conditions, cells were stimulated for 24 h at 37 °C, 5% CO_2. Cells were stained with anti-CD123-APC (clone AC145; Miltenyi), anti-CD304-PerCP-Cy-5.5 (clone 12C2; Biolegend), anti-CD11c-Pe-Cy7 (clone 3.9; eBioscience), anti-CD80-FITC (clone MAB104; Beckman Coulter), anti-CD86-V450 (clone 2331; BD) and anti-CD40-PE (MAB89; Beckman Coulter). Data was acquired on a Canto II (BD) and analyzed using Flowjo (version 10.1 Tree Star Inc). pDCs were defined as CD11c-CD123 + CD304 + within the lymphocyte gate as depicted in Fig. S1.

2.3. Statistical analysis

For statistical analysis flow cytometry-derived mean fluorescence intensities (MFI) were first log10-transformed and then fitted in linear mixed models in the R programming environment. Models included interaction terms between TLR effect and SNP, disease status (HBV or healthy) or sex and separate terms for sex, age and ethnicity. To test if the effect of TLR ligation was different between SNP genotypes, healthy individuals and HBV patients or between sexes, the model was fitted with and without these interaction terms. The effect on model fit was assessed using a likelihood ratio test (LRT). In case inclusion of interaction terms significantly improved model fit, regression coefficients and corresponding standard errors, p-values and adjusted p-values (corrected for multiple testing using the Holms procedure) were calculated from the model with that interaction term (Holm, 1979).

3. Results

3.1. Population prevalence of rs179008 genotypes

To assess the prevalence of the rs179008 variant across different ethnicities we genotyped a large number of healthy donors and CHB patients. The SNP was successfully genotyped in 176 (out of 231) healthy donors and 994 (out of 1054) CHB patients. First, we compared our data to that of a published large genome-wide screen on genetic variation across many different ethnic backgrounds (Auton et al., 2015). We assessed SNP prevalence between cohorts for the various ethnicities separately to exclude that the ethnical composition of the cohorts would influence SNP prevalence. The prevalence of the SNP variant (T) in our own healthy cohort (from Rotterdam and Antwerp; Healthy EA)) was nearly identical to that in the published database (Healthy DB) which contained rs179008 information of over 2000 individuals (Table 1; Fig. 1). In both cohorts the variant was most present in Caucasians (21–22% in males and 42–43% in females) and almost absent from Asians. The higher prevalence in females can be attributed to the X-chromosomal location of the TLR7 gene. (Table 1; Fig. 1). Overall, male CHB patients carried the variant with a comparable frequency as healthy males. Interestingly, the variant was significantly more prevalent in healthy Caucasian females (~42% in both cohorts) compared to Caucasian female CHB patients (28%). Heterozygous females were almost twofold more prevalent in the healthy cohorts (TA: 37% in healthy versus 21% in CHB). Thus, in Caucasian females the variant (T) allele may offer protection from CHB development.

3.2. Effect of rs179008 genotype on pDC phenotypic maturation

Next, we studied the effect of rs179008 genotype on pDC phenotypic maturation. Based on rs179008 genotype, sex, age and sample availability, we selected PBMCs for *in vitro* stimulation. We analyzed

Table 1
TLR7 rs179008 Genotype Distribution in Healthy individuals and HBV patients.

Gender	Ethnicity	Dataset	[b] A/AA (%)	AT	T/TT (%)	T (%)	vs HBV (RA) p-value[c]	vs Healthy (RA) p-value[c]
Male	Combined	Healthy (DB)[a]	1100 (89.2)	–	133 (10.8)	10.8	–	–
		Healthy (RA)[b]	50 (89.3)	–	6 (10.7)	10.7	–	–
		HBV (RA)	566 (85.8)	–	94 (14.2)	14.2	–	–
	Caucasian	Healthy (DB)	189 (78.8)		51 (21.3)	21.3	0.5192	1
		Healthy (RA)	21 (77.8)	–	6 (22.2)	22.2	0.6149	–
		HBV (RA)	256 (81.3)	–	59 (18.7)	18.7	–	0.6149
	Turkish	Healthy (DB)	–	–	–	–	–	–
		Healthy (RA)	–	–	–	–	–	–
		HBV (RA)	63 (86.3)	–	10 (13.7)	13.7	–	–
	Asian	Healthy (DB)	489 (97)		15 (3.0)	3.0	–	–
		Healthy (RA)	27 (100)	–	0 (0.0)	0.0	–	–
		HBV (RA)	133 (100)	–	0 (0.0)	0.0	–	–
	Other	Healthy (DB)	–	–	–		–	–
		Healthy (RA)	2 (100)	–	–	0.0	–	–
		HBV (RA)	114 (82.0)	–	25 (18.0)	18.0	–	–
Female	Combined	Healthy (DB)	989 (77.8)	251 (19.7)	31 (2.4)	22.2	**–**	–
		Healthy (RA)	95 (79.2)	22 (18.3)	3 (2.5)	20.8	–	–
		HBV (RA)	277 (82.9)	47 (14.1)	10 (3.0)	17.1	–	–
	Caucasian	Healthy (DB)	151 (57.4)	98 (37.3)	14 (5.3)	42.6	**0.0198**	1
		Healthy (RA)	33 (57.9)	21 (36.8)	3 (5.3)	42.1	0.101	–
		HBV (RA)	60 (72.3)	17 (20.5)	6 (7.2)	27.7	–	**0.0198**
	Turkish	Healthy (DB)	–	–	–	–	–	–
		Healthy (RA)	4 (80.0)	1 (20.0)	–	20.0	–	–
		HBV (RA)	31 (68.9)	11 (24.4)	3 (6.7)	31.1	–	–
	Asian	Healthy (DB)	476 (95.6)	21 (4.2)	1(0.2)	4.4	–	–
		Healthy (RA)	55 (100.0)	0 (0.0)	0 (0.0)	0.0	–	–
		HBV (RA)	121 (98.4)	2 (1.6)	0 (0.0)	1.6	–	–
	Other & Unknown	Healthy (DB)	–	–	–	–	–	–
		Healthy (RA)	3 (100)	–	–	0.0	–	–
		HBV (RA)	65 (78.3)	17 (20.5)	1 (1.2)	21.7	–	–

[a] Data from the "1000 genomes project" database (DB). Caucasians are all Europeans from this DB. East and south Asia were pooled.
[b] Data from healthy controls and HBV patients collected in Rotterdam and Antwerp (RA).
[c] Prevalence of variant allele carrying individuals in the two cohorts being compared by Fisher exact test.

PBMC samples from 20 healthy individuals (10F/10M) carrying the WT allele only (A or AA), and 10 heterozygous (TA) females. Due to the low prevalence of the variant, we only had available PBMCs of 8 healthy individuals (2F/6M) homo- or hemizygous for the variant (T or TT; Table S1). WT individuals were chosen to match the age of those carrying the variant.

After thawing, PBMCs were cultured in the presence or absence of TLR-ligands Loxoribin (Lox; TLR7), R848 (TLR7 & TLR8) and CpG (TLR9) and after 24 h the levels of CD40, CD80 and CD86 were assessed by flow cytometry (Fig. S1). Lox, R848 and CpG all significantly increased expression levels of one or more surface markers (Fig. 2A). Percentages of positive cells were also increased, but skewed towards "all positive" (Fig. S2). Therefore we used expression levels for further statistical analysis. Lox and R848 potently induced CD40 and CD86, while CpG hardly induced CD86, but upregulated CD40 and CD80. Lox, but not R848, also induced expression of CD80. Plotting samples from the two sexes separately indicated that CD40 and CD80 expression levels were significantly higher in females and this was most clear after TLR7/8 ligation (Fig. 2B). In contrast, expression levels of CD86 were higher in males, both at baseline and after TLR9 ligation. Next, to examine the effect of rs179008 genotype on TLR-ligand induced pDC maturation we fitted linear mixed models including and excluding SNP genotype information and determined the effect on model fit using likelihood ratio tests (LRT). In this analysis samples from one individual were matched and age and sex were included in the models to reduce confounding effects. LRTs indicated that both CD40 and CD86, but not CD80, upregulation (i.e. expression change relative to subject-matched cells cultured without TLR ligands) was influenced by rs179008 genotype (Table 2). Closer inspection revealed that pDCs from heterozygous (TA) females significantly more upregulated CD40 and CD86 upon TLR 7/8 ligation, compared to WT individuals (A and AA; Table 2, Fig. 3). Homo- or hemizygous presence of the variant allele (TT or T) did not appear to affect CD40 and CD86 upregulation compared to WT, but for these genotypes samples were limited. CpG-induced pDC maturation was less influenced by the SNP (Table 2; Fig. 3). Together these data indicate that TLR7/8 induced maturation of pDCs from females in general, but especially of rs179008 heterozygous females, was augmented.

3.3. Effect of sex on pDC phenotypic maturation

Next, we investigated the effect of sex on pDC phenotypic maturation. Regression analysis demonstrated for all three co-stimulatory receptors that model fit was significantly improved by including the interaction between sex and TLR-effect (Table 3). All receptors were upregulated more on female pDCs, but only upon TLR7/8 ligation (Table 3 and Fig. 4). Because the rs179008 heterozygous genotype (TA) only occurs in females, SNP genotype will contribute to, or could even explain, this effect of sex. Directly comparing pDCs from homo- and heterozygous females (AA vs TA; Fig. 3 red symbols only) as well as comparing males and females carrying only the WT allele (A vs AA; Fig. 3 black versus red) suggested that the higher upregulation of CD40 in females may be fully attributed to the SNP, while for CD86 the SNP may not be fully responsible. For CD80, as previously indicated, the SNP does not seem to contribute at all (Table 2). To further isolate the sex from the SNP effect, we performed regression analysis on males and

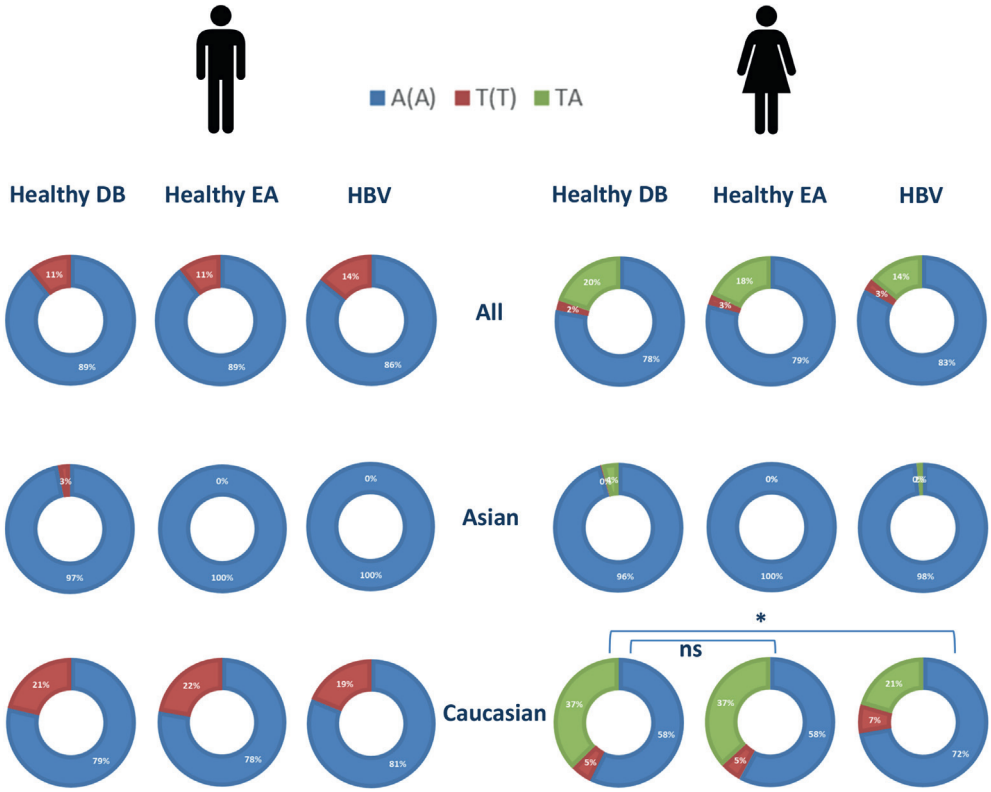

Fig. 1. TLR7 rs179008 Genotype Distribution in Healthy individuals and HBV patients. Visualization of the most important findings in Table 1.

females carrying the WT allele only (A versus AA; blue symbols in Fig. 4). For CD80 and CD86 regression analysis indicated a remaining effect of sex on model fit within SNP WT samples. Closer inspection revealed that an interaction between sex and TLR7/8 ligand treatment remained also in this patient sub-group, but was reduced to a trend ($p < 0.1$) after applying multiple testing correction (Table 4). CD40 upregulation within the SNP WT samples was not affected by sex.

In summary, pDCs from females more profoundly upregulated all maturation markers upon TLR7/8 ligation, which for CD40, and to a lesser extend for CD86, could be attributed to the rs179008 TA genotype.

3.4. Effect of CHB on pDC phenotypic maturation

Lastly, we assessed pDC maturation in CHB patients. We retrieved PBMC samples from 28 CHB patients, 19 (10F/9M) carrying the WT genotype only, 6 heterozygous females and 3 carrying the variant only (1F/2M). While the healthy cohort consisted mostly of Caucasians, approximately half of our CHB cohort was of Turkish decent, including most females (Table S1). The imbalanced distribution of ethnicities over sexes did not allow proper evaluation of the effect of sex or rs179008 genotype within this cohort (Table S1). In CHB patients, however, we observed a rather low induction of maturation markers, especially of CD40 (Fig. 5A). Direct comparison of the healthy and CHB cohorts by regression analysis, taking into account sex and ethnicity, suggested that CD40 upregulation by TLR7 ligands may indeed be impaired in CHB patients (Fig. 5B and Table 5). Inspection of viral and patient parameters (HBsAg, HBV DNA, ALT) did not reveal any causal relation between these parameters and this impairment (data not shown).

4. Discussion

Our results demonstrate that rs179008 may reduce the risk of heterozygous Caucasian females to develop CHB, and that the heterozygous SNP genotype and female sex together positively influence pDC phenotypic maturation, while CHB impairs pDC maturation.

Our data adds to previous reports on the influence of sex on production of IFNα by pDCs, demonstrating that also surface maturation markers are upregulated more in female pDCs (Berghöfer et al., 2006; Meier et al., 2009; Seillet et al., 2012, 2013). The effect of sex on upregulation of especially CD40 but also CD86 was respectively mostly or partially explained by the rs179008 variant allele. For CD80 only an effect of sex was observed that was independent of rs179008 genotype.

Although we applied stringent statistical criteria to reach our conclusions, the limited number of samples in our study may have caused us to miss less pronounced effects. Furthermore, analysis of the effect of sex and rs179008 in CHB patients was hampered by differences in ethnicities between the sexes in this cohort. For more definitive conclusions on TLR7 function in CHB patients, therefore, additional studies are needed.

Our observations that the rs179008 variant may protect against CHB in Caucasian females is contrasting previous findings for HCV and HIV. In both infections, the variant allele associated with higher disease prevalence and augmented disease progression, and with reduced response to IFNα-therapy for HCV (Oh et al., 2009; Said et al., 2014; Askar et al., 2010; Schott et al., 2007b; Fakhir et al., 2018). In all these studies, effects were most prominent in (mostly rs179008 heterozygous) females and all studies predominantly contained Caucasian individuals. The fact that HCV and HIV, but not HBV, have been reported to activate TLR7 and pDCs, could contribute to the contrasting observations for these diseases (Woltman et al., 2011; Beignon et al., 2005; Takahashi et al., 2010). To confirm that rs179008 SNP is truly protective for CHB, our study needs to be repeated in a larger cohort. This cohort preferably should also contain patients spontaneously clearing the disease, similar to a recent study performed on TLR9 SNPs in HCV disease progression (Fischer et al., 2017).

In HCV, the rs179008 variant associated with lower levels of IL-10 and Type III IFNs in infected liver tissue, while healthy donor pDCs carrying the variant displayed impaired IFNα production (Oh et al.,

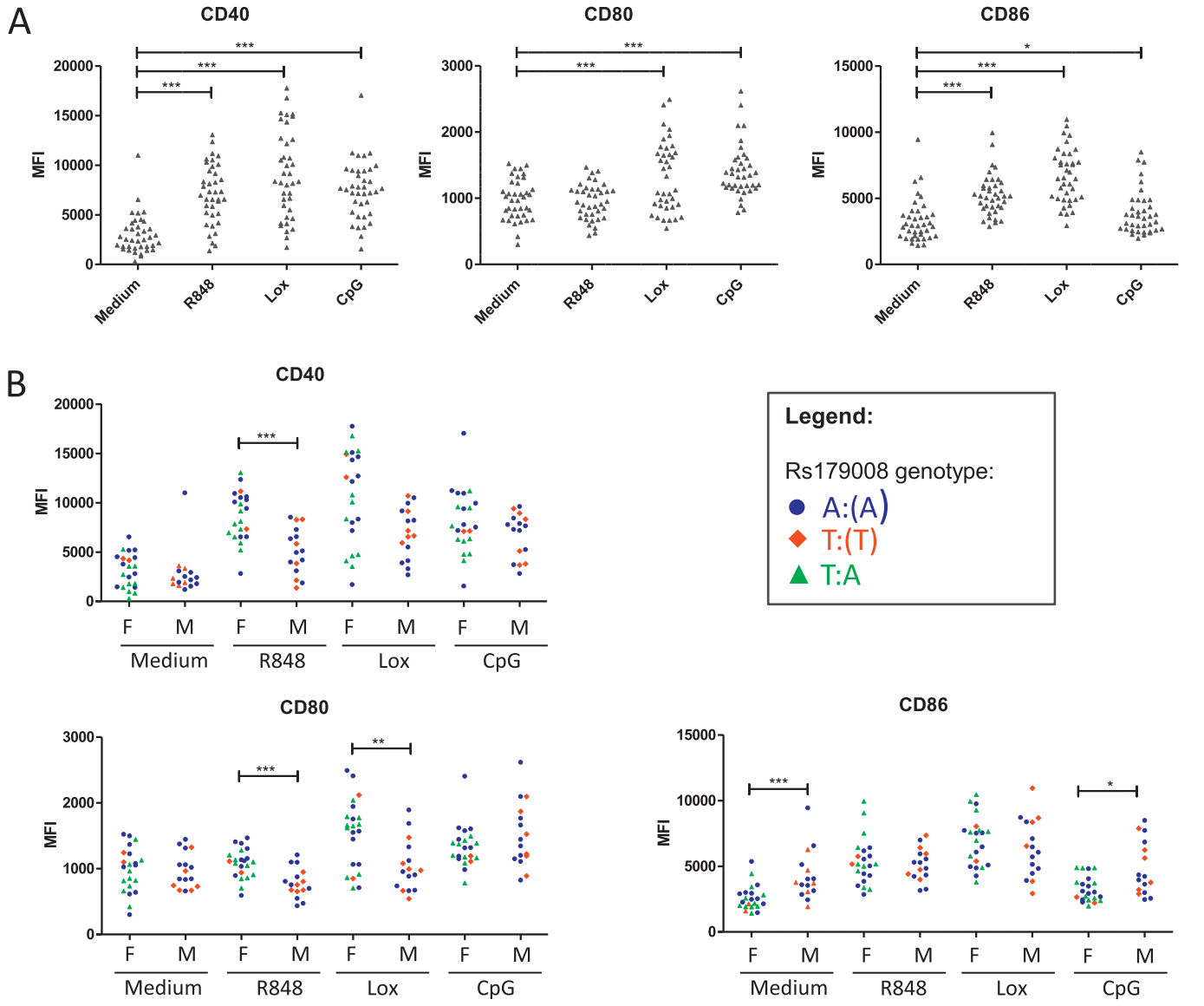

Fig. 2. Effect of the rs179008 SNP on the expression level of maturation markers on pDCs from healthy donors. (A)Mean fluorescent intensities (MFI) of CD40, CD80 and CD86 after 24 h in the presence of absence of indicated TLR-ligands or medium alone (B) Data as in A but for males (M) and females (F) separately and color coded for the rs179008 TLR7 SNP. P-values *** < 0.001, ** < 0.01 and * < 0.05 by paired t-tests comparing TLR-ligand activated pDCs to medium cultured pDCs (A) or by unpaired t-tests comparing pDCs from males to those of females for each condition (B), all test were performed on log transformed data. Non-significant results are not shown. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2009; Askar et al., 2010). These are the only reported effects of the SNP at the protein/cellular level and these data were mostly (for livers), or completely (for pDCs), derived from hemizygous males. As stated above, however, most reported clinical effects of the SNP are observed in females (Oh et al., 2009; Schott et al., 2007b). We also identified effects of the SNP only in females. Unfortunately, we could not assess IFNα secretion to reunite these studies, because we relied on pDCs from a biobank and IFNα secretion is hampered by cryopreservation (Ida et al., 2006).

Incomplete X-inactivation of TLR7 may contribute to the effects of sex. Although previous studies found no evidence for incomplete or skewed X-inactivation this was recently challenged by Souyris and colleagues who reported that pDC express more TLR7 in females due to escape of X-inactivation (Schott et al., 2007a; Berghöfer et al., 2006; Souyris et al., 2018). Besides, TLR7 function may be enhanced by female hormones and/or enhanced expression of molecules downstream of TLR7 (Seillet et al., 2012, 2013; Griesbeck et al., 2015). It remains unclear why possession of a heterozygous genotype offers an advantage when it comes to pDC maturation and reducing CHB risk. Possibly, pDC heterogeneity may facilitate their diverse functions in innate and adaptive immunity.

Because we stimulated PBMCs rather than pDCs, also other immune cells contributed to pDC maturation via cytokines or membrane receptors, as would occur *in vivo*. Previously, we reported on indirect effects of other cells in culture (Woltman et al., 2011; van der Aa et al., 2015). TLR7/8 ligand R848 also activates monocytes and other DC-subsets expressing TLR8 (or TLR7 upon activation) which indirectly contributes to the activation of pDCs (Schreibelt et al., 2010; Hou et al., 2014; Giltiay et al., 2016). Dissecting direct and indirect effects requires additional experiments with sorted pDCs, which are not feasible due to insufficient fresh PBMC of rs179008 genotyped individuals.

Recently, two studies reported that pDCs sorted based on the absence of CD11c and expression of CD123 and BDCA4, as was also our strategy and common practice until recently, are contaminated by a

Table 2
Interaction of TLR7 SNP rs179008 with TLR-ligand induced pDC maturation in healthy individuals.

		CD40			CD80			CD86		
rs179008 LRT p- value:		0.0003			0.2465			0.0082		
Type	Term	Value	p	adj.p	Value	p	adj.p	Value	p	adj.p
Main effects	Intercept	3.38			2.98			3.46		
	CpG	0.40	0	**0**	0.17	0	**0**	0.04	0.2702	1
	Lox	0.42	0	**0**	0.12	0.0001	**0.0005**	0.26	0	**0**
	R848	0.34	0	**0**	0.00	0.8678	1	0.15	0.0002	**0.0027**
	rs179008 TT	0.03	0.7221	1	−0.03	0.4586	1	−0.06	0.3897	1
	rs179008 TA	−0.34	0.0009	**0.0083**	−0.05	0.2159	0.8635	−0.11	0.0784	0.6268
	SexM	−0.20	0.0096	0.0671	−0.08	0.0366	0.1830	0.09	0.0460	0.4137
	Age	0.00	0.0785	0.3926	0.00	0.4712	1	0.00	0.7196	1
Interactions	CpG: rs179008 TT	−0.03	0.7356	1	–	–	–	0.06	0.3835	1
	Lox: rs179008 TT	0.09	0.2728	1	–	–	–	0.05	0.4535	1
	R848: rs179008 TT	−0.08	0.3737	1	–	–	–	0.09	0.1932	1
	CpG: rs179008 TA	0.20	0.0129	0.0773	–	–	–	0.10	0.1369	0.9582
	Lox: rs179008 TA	0.26	0.0013	**0.0103**	–	–	–	0.23	0.0010	**0.0101**
	R848: rs179008 TA	0.32	0.0001	**0.0010**	–	–	–	0.23	0.0007	**0.0082**

Main effects and interactions significant ($p < 0.05$) after applying multiple testing correction are in bold.

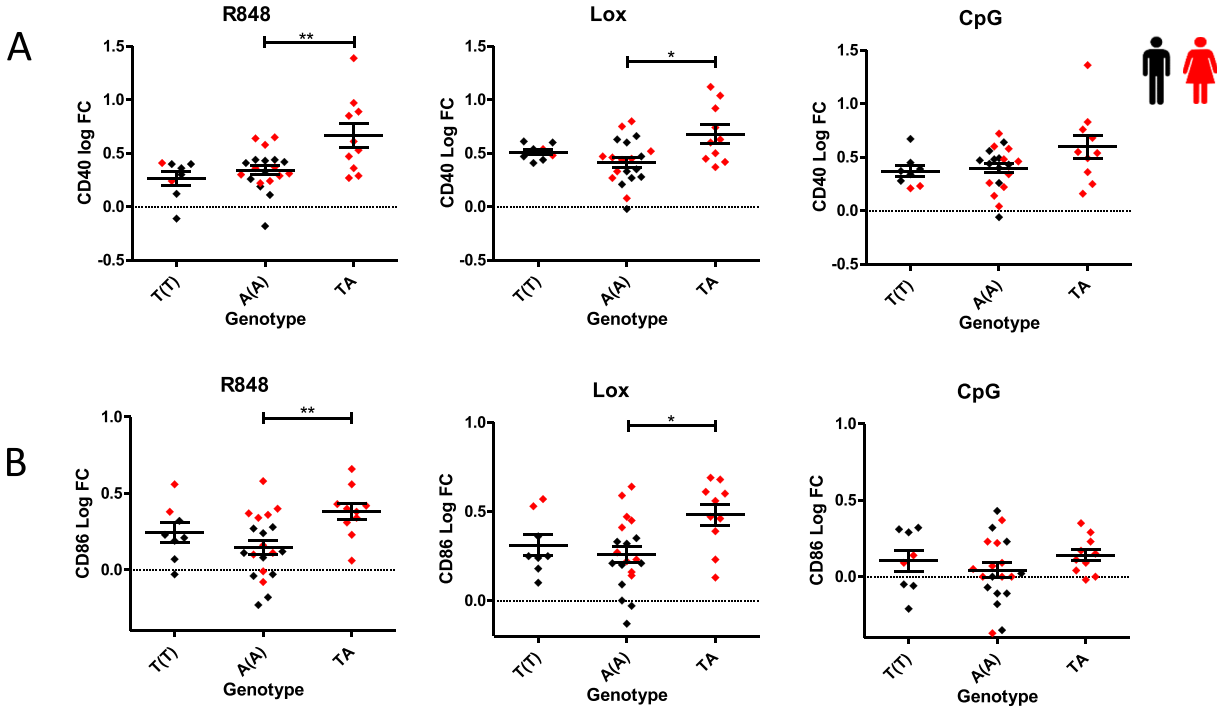

Fig. 3. Interaction of SNP rs179008 with TLR induced upregulation of maturation markers on pDCs from healthy donors. (A & B) Fold change (FC) in log mean fluorescence intensity of CD40 (A) and CD86 (B) on pDCs from healthy donors upon TLR ligation compared to incubation with medium only (i.e. log(TLR)-log (medium)). Data was grouped according to TLR rs179008 genotype and colored according to sex of the donor (males in black, females in red). P-values displayed represent the (multiple testing corrected) adjusted p-values displayed in Table 2 for the interaction between the genotypes and TLR-ligand induced upregulation of each surface marker. **$p < 0.01$, *$p < 0.05$. Non-significant results are not shown. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

population of pre-cDCs expressing AXL that highly expresses co-stimulatory molecules (See et al., 2017; Villani et al., 2017). Although it can be deduced from these studies that *bona fide* pDCs still outnumber contaminating cDCs using this gating strategy, it is not known whether the extent of pre-cDC contamination varies between males and females or whether it is affected by infection. Therefore, we do not know if pre-cDCs have influenced our results. Future studies should address this issue.

Recently, oral TLR7 ligands successfully induced viral control in Woodchuck and chimpanzee HBV-animal models (Menne et al., 2015; Lanford et al., 2013). Disappointingly in humans, oral TLR7 ligands thus far have not achieved any beneficial effect, despite that treatment was well tolerated (Gane et al., 2015; Janssen et al., 2017). It is not clear why clinical benefit in CHB patients was lacking, but impaired patient TLR7 responses could have contributed. Of note, these studies mainly included Asians and males and therefore only very few rs179008 variant carrying individuals and even less to no SNP heterozygous females were treated.

Future studies are needed to determine the functional consequences of the variation in pDC maturation. Effects were most prominent for

Table 3
Interaction of sex with TLR-ligand induced pDC maturation in healthy individuals.

		CD40			CD80			CD86		
Sex LRT p- value:		0.0039			0.0001			0		
	Term	Value	p	adj.p	Value	p	adj.p	Value	p	adj.p
Main effects	Intercept	3.25			2.93			3.37		
	CpG	0.47	0	**0**	0.15	0	**0.0002**	0.11	0.0026	**0.0077**
	Lox	0.56	0	**0**	0.20	0	**0**	0.43	0	**0**
	R848	0.51	0	**0**	0.06	0.0838	0.3353	0.32	0	**0**
	SexM	−0.02	0.8177	0.8818	0.01	0.7935	1	0.21	0.0001	**0.0004**
	Age	0.00	0.1291	0.3872	0.00	0.8484	1	0.00	0.6595	0.6595
Interactions	CpG: Sex M	−0.05	0.4409	0.8818	0.03	0.6066	1	−0.06	0.2919	0.5838
	Lox: Sex M	−0.13	0.0569	0.2276	−0.18	0.0014	**0.0082**	−0.25	0	**0**
	R848: Sex M	−0.24	0.0008	**0.0041**	−0.16	0.0045	**0.0225**	−0.22	0.0001	**0.0003**

Main effects and interactions significant ($p < 0.05$) after applying multiple testing correction are in bold.

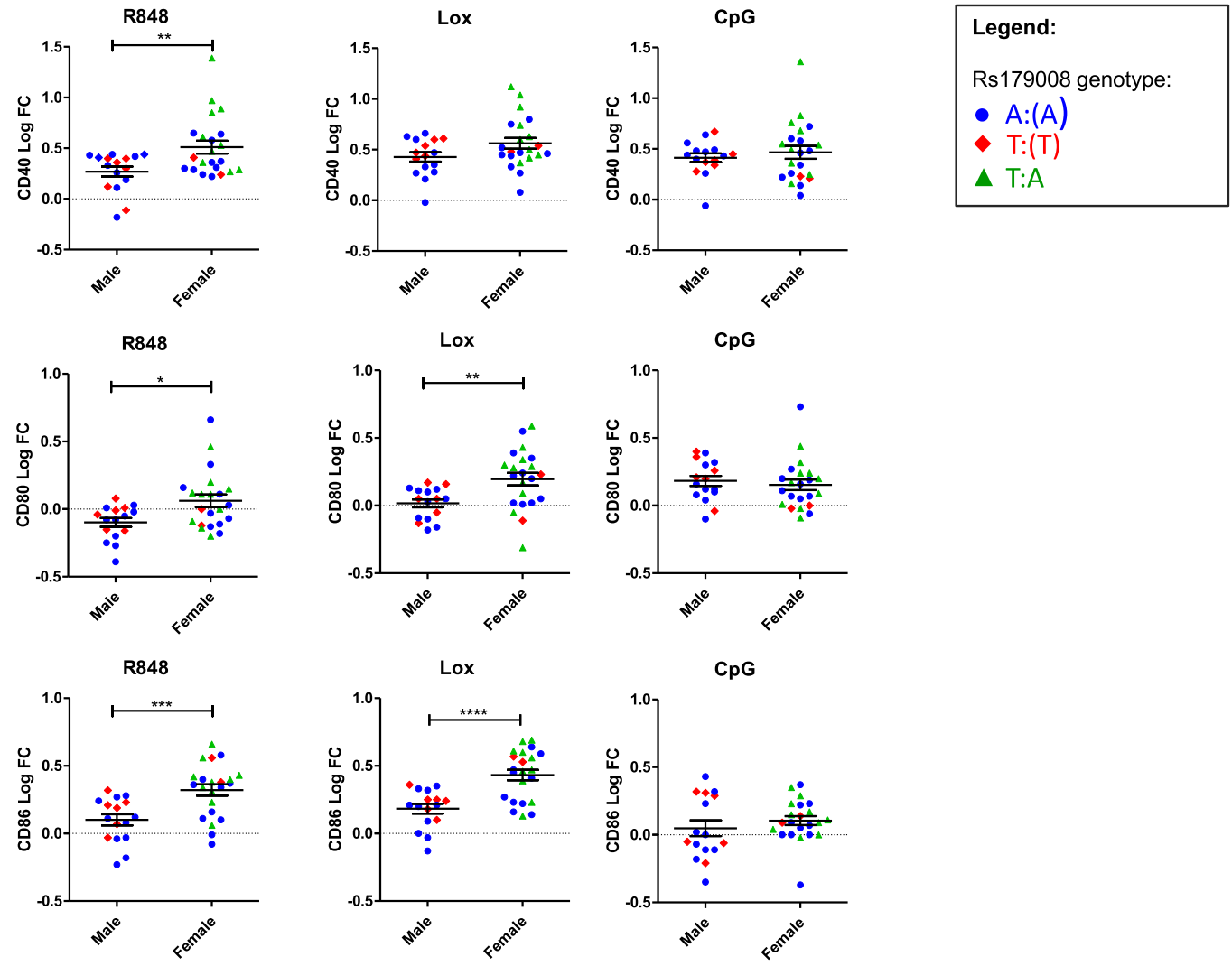

Fig. 4. Interaction of sex with TLR-ligand induced pDC surface marker upregulation. Fold change (FC) in log mean fluorescence intensity of CD40, CD80 and CD86 on pDCs from healthy donors upon TLR ligation compared to incubation with medium only (i.e. log(TLR)-log (medium)). Data are grouped according to sex and colored according to rs79008 SNP (Legend). P-values displayed represent the (multiple testing corrected) adjusted p-values displayed in Table 3 for the interaction between sex and TLR-ligand induced upregulation of each surface marker. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Non-significant results are not shown.

Table 4
Interaction of Sex with TLR-ligand induced pDC maturation in healthy individuals carrying the WT A(A) rs179008 allele only.

		CD40			CD80			CD86		
Sex LRT p-value in A & AA only:		0.2352			0.0073			0.0152		
	Term	Value	p	adj.p	Value	p	adj.p	Value	p	adj.p
Main effects	Intercept	3.49			2.94			3.46		
	CpG	0.40	0	**0**	0.18	0.0019	**0.0133**	0.07	0.2182	0.6545
	Lox	0.42	0	**0**	0.20	0.0005	**0.0040**	0.36	0	**0**
	R848	0.34	0	**0**	0.08	0.1746	0.6983	0.23	0.0001	**0.0004**
	SexM	−0.18	0.0831	0.1663	0.04	0.6106	1	0.18	0.0098	0.0500
	Age	0.00	0.7225	0.7225	0.00	0.6928	1	−0.00	0.7572	1
Interactions	CpG: Sex M	–	–	–	0.03	0.7258	1	−0.05	0.5318	1
	Lox: Sex M	–	–	–	−0.20	0.0118	0.0692	−0.21	0.0083	0.0500
	R848: Sex M	–	–	–	−0.20	0.0115	0.0692	−0.17	0.0263	0.1051

Main effects and interactions significant ($p < 0.05$) after applying multiple testing correction are in bold.

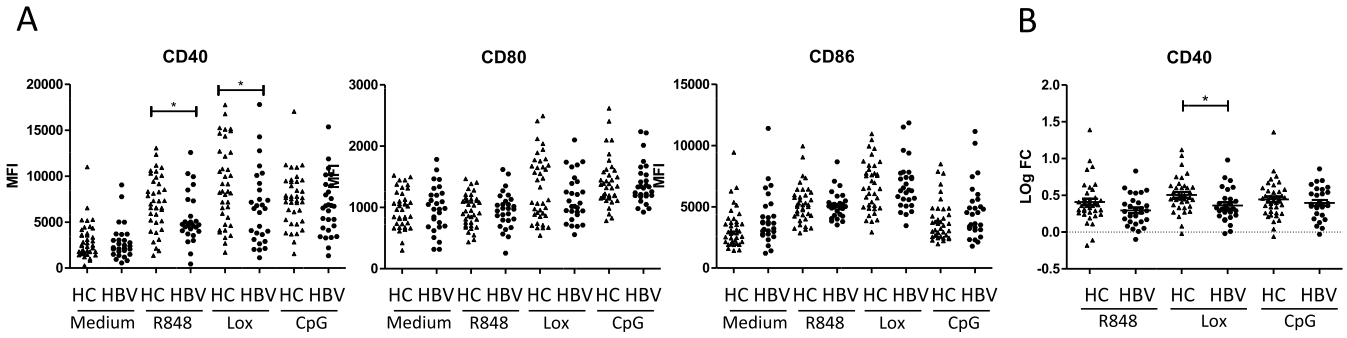

Fig. 5. Effect of TLR ligation on maturation markers on pDCs from HBV patients and healthy individuals. A)Mean fluorescent intensities (MFI) of CD40, CD80 and CD86 after 24 h in the presence of absence of indicated TLR-ligands or medium alone for healthy individual (HC) and HBV patient-derived pDCs. P-values comparing healthy and HBV for all conditions by unpaired t-tests on log transformed data. *$p < 0.05$. Non-significant p-values are not shown (B) Fold change (FC) in log mean fluorescence intensity of CD40 on pDCs from healthy donors and HBV patient upon TLR ligation compared to incubation with medium only (i.e. log(TLR)-log (medium)). *$p < 0.05$ represents adjusted p-values displayed in Table 4 for the interaction between HBV status Lox induced upregulation of CD40.

Table 5
Interaction between chronic HBV infection and TLR-ligand induced pDC maturation.

		CD40			CD80			CD86		
HBV LRT p-value:		0.027			0.7405			0.2298		
	Term	Value	p	adj.p	Value	p	adj.p	Value	p	adj.p
Main effects	Intercept	3.41			2.96			3.46		
	CpG	0.44	0	**0**	0.17	0	**0**	0.08	0.0013	**0.0064**
	Lox	0.50	0	**0**	0.10	0	**0.0001**	0.30	0	**0**
	R848	0.41	0	**0**	0.00	0.9490	1	0.19	0	**0**
	CHB	−0.06	0.4737	1	0.00	0.9168	1	0.07	0.0430	0.1720
	Sex M	−0.12	0.0423	0.2115	−0.08	0.0020	**0.0098**	0.05	0.1204	0.3613
	Age	0.00	0.7110	1	0.00	0.1975	0.7901	0.00	0.7040	0.7040
	Ethnicity Turkish	0.05	0.5726	1	−0.05	0.2085	0.7901	−0.06	0.1520	0.3613
Interactions	CpG: CHB	−0.05	0.3765	1	–	–	–	–	–	–
	Lox: CHB	−0.14	0.0071	**0.0498**	–	–	–	–	–	–
	R848: CHB	−0.11	0.0312	0.1873	–	–	–	–	–	–

Main effects and interactions significant ($p < 0.05$) after applying multiple testing correction are in bold.

CD40, which is mostly expressed on antigen presenting cells. Its ligand is expressed on activated T cells, endothelial cells and platelets (reviewed in (Elgueta et al., 2009)). On pDCs, CD40 ligation has been described to augment the production of IL-6, thereby facilitating generation of antibody producing plasma cells (Jego et al., 2003). Furthermore, CD40 signaling can aid effective activation of T cells or prevent T cell exhaustion (Krug et al., 2001; Fonteneau et al., 2003; Tel et al., 2013; Fuse et al., 2009; Isogawa et al., 2013). In CHB patients low and exhausted T cells are consistently observed and held responsible for the inability to clear HBV (Ferrari et al., 1990; Jung et al., 1991; Boni et al., 2007, 2012; Bertoletti and Ferrari, 2016). In CHB patients and individuals homozygous for rs179008 less induction of CD40 may thus affect the ability of pDCs to drive adaptive immune responses and could facilitate T cell exhaustion. Simultaneous low induction of CD86 and CD80 may further limit adaptive responses.

Taken together, our study indicates that rs179008 genotype may be relevant for the immune response against HBV and highlights that TLR7 induced pDC maturation is affected by sex, TLR7 genotypic variation and chronic viral infection. These findings provide important insight in the variation in TLR7 responses in healthy and diseased individuals which is of relevance for the further clinical development and evaluation of TLR7-based therapies.

Conflicts of interest

AB has been consulting or in advisory boards for Gilead Sciences and Bristol-Myers Squibb and has received research grants from Roche, Gilead Sciences, Fujirebio, and Janssen. AW has received research grants from Roche.

Acknowledgements

This investigator-initiated study was supported by an unrestricted grant from Roche and the Virgo consortium, funded by the Dutch government project number FES0908. The authors wish to acknowledge all HBV 99-01 and PARC study group members for their help in data and material acquisition. Viral hepatitis outreach screening events in Antwerp, Belgium were supported by funding from the Region of Flanders through the Centre for Medical Innovation and grants from Gilead Life Sciences; Bristol-Myers Squibb and Sandoz. TV is supported by a Research Mandate of the Foundation Against Cancer Belgium, No. 2014-087. AW is supported by a ZonMW VIDI grant project number 91712329.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.antiviral.2018.06.015.

References

Askar, E., Ramadori, G., Mihm, S., 2010. Toll-like receptor 7 rs179008/Gln11Leu gene variants in chronic hepatitis C virus infection. J. Med. Virol. 82, 1859–1868.

Auton, A., Abecasis, G.R., Altshuler, D.M., Durbin, R.M., Abecasis, G.R., Bentley, D.R., Chakravarti, A., Clark, A.G., Donnelly, P., Eichler, E.E., Flicek, P., Gabriel, S.B., Gibbs, R.A., Green, E.D., Hurles, M.E., Knoppers, B.M., Korbel, J.O., Lander, E.S., Lee, C., Lehrach, H., Mardis, E.R., Marth, G.T., McVean, G.A., Nickerson, D.A., Schmidt, J.P., Sherry, S.T., Wang, J., Wilson, R.K., Gibbs, R.A., Boerwinkle, E., Doddapaneni, H., Han, Y., Korchina, V., Kovar, C., Lee, S., Muzny, D., Reid, J.G., Zhu, Y., Wang, J., Chang, Y., Feng, Q., Fang, X., Guo, X., Jian, M., Jiang, H., Jin, X., Lan, T., Li, G., Li, J., Li, Y., Liu, S., Liu, X., Lu, Y., Ma, X., Tang, M., Wang, B., Wang, G., Wu, H., Wu, R., Xu, X., Yin, Y., Zhang, D., Zhang, W., Zhao, J., Zhao, M., Zheng, X., Lander, E.S., Altshuler, D.M., Gabriel, S.B., Gupta, N., Gharani, N., Toji, L.H., Gerry, N.P., Resch, A.M., Flicek, P., Barker, J., Clarke, L., Gil, L., Hunt, S.E., Kelman, G., Kulesha, E., Leinonen, R., McLaren, W.M., Radhakrishnan, R., Roa, A., Smirnov, D., Smith, R.E., Streeter, I., Thormann, A., Toneva, I., Vaughan, B., Zheng-Bradley, X., Bentley, D.R., Grocock, R., Humphray, S., James, T., Kingsbury, Z., Lehrach, H., Sudbrak, R., Albrecht, M.W., Amstislavskiy, V.S., Borodina, T.A., Lienhard, M., Mertes, F., Sultan, M., Timmermann, B., Yaspo, M.-L., Mardis, E.R., Wilson, R.K., Fulton, L., Fulton, R., Sherry, S.T., Ananiev, V., Belaia, Z., Beloslyudtsev, D., Bouk, N., Chen, C., Church, D., Cohen, R., Cook, C., Garner, J., Hefferon, T., Kimelman, M., Liu, C., Lopez, J., Meric, P., O'Sullivan, C., Ostapchuk, Y., Phan, L., Ponomarov, S., Schneider, V., Shekhtman, E., Sirotkin, K., Slotta, D., Zhang, H., McVean, G.A., Durbin, R.M., Balasubramaniam, S., Burton, J., Danecek, P., Keane, T.M., Kolb-Kokocinski, A., McCarthy, S., Stalker, J., Quail, M., Schmidt, J.P., Davies, C.J., Gollub, J., Webster, T., Wong, B., Zhan, Y., Auton, A., Campbell, C.L., Kong, Y., Marcketta, A., Gibbs, R.A., Yu, F., Antunes, L., Bainbridge, M., Muzny, D., Sabo, A., Huang, Z., Wang, J., Coin, L.J.M., Fang, L., Guo, X., Jin, X., Li, G., Li, Q., Li, Y., Li, Z., Lin, H., Liu, B., Luo, R., Shao, H., Xie, Y., Ye, C., Yu, C., Zhang, F., Zheng, H., Zhu, H., Alkan, C., Dal, E., Kahveci, F., Marth, G.T., Garrison, E.P., Kural, D., Lee, W.-P., Fung Leong, W., Stromberg, M., Ward, A.N., Wu, J., Zhang, M., Daly, M.J., DePristo, M.A., Handsaker, R.E., Altshuler, D.M., Banks, E., Bhatia, G., del Angel, G., Gabriel, S.B., Genovese, G., Gupta, N., Li, H., Kashin, S., Lander, E.S., McCarroll, S.A., Nemesh, J.C., Poplin, R.E., Yoon, S.C., Lihm, J., Makarov, V., Clark, A.G., Gottipati, S., Keinan, A., Rodriguez-Flores, J.L., Korbel, J.O., Rausch, T., Fritz, M.H., Stütz, A.M., Flicek, P., Beal, K., Clarke, L., Datta, A., Herrero, J., McLaren, W.M., Ritchie, G.R.S., Smith, R.E., Zerbino, D., Zheng-Bradley, X., Sabeti, P.C., Shlyakhter, I., Schaffner, S.F., Vitti, J., Cooper, D.N., Ball, E.V., Stenson, P.D., Bentley, D.R., Barnes, B., Bauer, M., Keira Cheetham, R., Cox, A., Eberle, M., Humphray, S., Kahn, S., Murray, L., Peden, J., Shaw, R., Kenny, E.E., Batzer, M.A., Konkel, M.K., Walker, J.A., MacArthur, D.G., Lek, M., Sudbrak, R., Amstislavskiy, V.S., Herwig, R., Mardis, E.R., Ding, L., Koboldt, D.C., Larson, D., Ye, K., Gravel, S., Swaroop, A., Chew, E., Lappalainen, T., Erlich, Y., Gymrek, M., Frederick Willems, T., Simpson, J.T., Shriver, M.D., Rosenfeld, J.A., Bustamante, C.D., Montgomery, S.B., De La Vega, F.M., Byrnes, J.K., Carroll, A.W., DeGorter, M.K., Lacroute, P., Maples, B.K., Martin, A.R., Moreno-Estrada, A., Shringarpure, S.S., Zakharia, F., Halperin, E., Baran, Y., Lee, C., Cerveira, E., Hwang, J., Malhotra, A., Plewczynski, D., Radew, K., Romanovitch, M., Zhang, C., Hyland, F.C.L., Craig, D.W., Christoforides, A., Homer, N., Izatt, T., Kurdoglu, A.A., Sinari, S.A., Squire, K., Sherry, S.T., Xiao, C., Sebat, J., Antaki, D., Gujral, M., Noor, A., Ye, K., Burchard, E.G., Hernandez, R.D., Gignoux, C.R., Haussler, D., Katzman, S.J., James Kent, W., Howie, B., Ruiz-Linares, A., Dermitzakis, E.T., Devine, S.E., Abecasis, G.R., Min Kang, H., Kidd, J.M., Blackwell, T., Caron, S., Chen, W., Emery, S., Fritsche, L., Fuchsberger, C., Jun, G., Li, B., Lyons, R., Scheller, C., Sidore, C., Song, S., Sliwerska, E., Taliun, D., Tan, A., Welch, R., Kate Wing, M., Zhan, X., Awadalla, P., Hodgkinson, A., Li, Y., Shi, X., Quitadamo, A., Lunter, G., McVean, G.A., Marchini, J.L., Myers, S., Churchhouse, C., Delaneau, O., Gupta-Hinch, A., Kretzschmar, W., Iqbal, Z., Mathieson, I., Menelaou, A., Rimmer, A., Xifara, D.K., Oleksyk, T.K., Fu, Y., Liu, X., Xiong, M., Jorde, L., Witherspoon, D., Xing, J., Eichler, E.E., Browning, B.L., Browning, S.R., Hormozdiari, F., Sudmant, P.H., Khurana, E., Durbin, R.M., Hurles, M.E., Tyler-Smith, C., Albers, C.A., Ayub, Q., Balasubramaniam, S., Chen, Y., Colonna, V., Danecek, P., Jostins, L., Keane, T.M., McCarthy, S., Walter, K., Xue, Y., Gerstein, M.B., Abyzov, A., Balasubramanian, S., Chen, J., Clarke, D., Fu, Y., Harmanci, A.O., Jin, M., Lee, D., Liu, J., Jasmine Mu, X., Zhang, J., Zhang, Y., Li, Y., Luo, R., Zhu, H., Alkan, C., Dal, E., Kahveci, F., Marth, G.T., Garrison, E.P., Kural, D., Lee, W.-P., Ward, A.N., Wu, J., Zhang, M., McCarroll, S.A., Handsaker, R.E., Altshuler, D.M., Banks, E., del Angel, G., Genovese, G., Hartl, C., Li, H., Kashin, S., Nemesh, J.C., Shakir, K., Yoon, S.C., Lihm, J., Makarov, V., Degenhardt, J., Korbel, J.O., Fritz, M.H., Meiers, S., Raeder, B., Rausch, T., Stütz, A.M., Flicek, P., Paolo Casale, F., Clarke, L., Smith, R.E., Stegle, O., Zheng-Bradley, X., Bentley, D.R., Barnes, B., Keira Cheetham, R., Eberle, M., Humphray, S., Kahn, S., Murray, L., Shaw, R., Lameijer, E.-W., Batzer, M.A., Konkel, M.K., Walker, J.A., Ding, L., Hall, I., Ye, K., Lacroute, P., Lee, C., Cerveira, E., Malhotra, A., Hwang, J., Plewczynski, D., Radew, K., Romanovitch, M., Zhang, C., Craig, D.W., Homer, N., Church, D., Xiao, C., Sebat, J., Antaki, D., Bafna, V., Michaelson, J., Ye, K., Devine, S.E., Gardner, E.J., Abecasis, G.R., Kidd, J.M., Mills, R.E., Dayama, G., Emery, S., Jun, G., Shi, X., Quitadamo, A., Lunter, G., McVean, G.A., Chen, K., Fan, X., Chong, Z., Chen, T., Witherspoon, D., Xing, J., Eichler, E.E., Chaisson, M.J., Hormozdiari, F., Huddleston, J., Malig, M., Nelson, B.J., Sudmant, P.H., Parrish, N.F., Khurana, E., Hurles, M.E., Blackburne, B., Lindsay, S.J., Ning, Z., Walter, K., Zhang, Y., Gerstein, M.B., Abyzov, A., Chen, J., Clarke, D., Lam, H., Jasmine Mu, X., Sisu, C., Zhang, J., Zhang, Y., Gibbs, R.A., Yu, F., Bainbridge, M., Challis, D., Evani, U.S., Kovar, C., Lu, J., Muzny, D., Nagaswamy, U., Reid, J.G., Sabo, A., Yu, J., Guo, X., Li, W., Li, Y., Wu, R., Marth, G.T., Garrison, E.P., Fung Leong, W., Ward, A.N., del Angel, G., DePristo, M.A., Gabriel, S.B., Gupta, N., Hartl, C., Poplin, R.E., Clark, A.G., Rodriguez-Flores, J.L., Flicek, P., Clarke, L., Smith, R.E., Zheng-Bradley, X., MacArthur, D.G., Mardis, E.R., Fulton, R., Koboldt, D.C., Gravel, S., Bustamante, C.D., Craig, D.W., Christoforides, A., Homer, N., Izatt, T., Sherry, S.T., Xiao, C., Dermitzakis, E.T., Abecasis, G.R., Min Kang, H., McVean, G.A., Gerstein, M.B., Balasubramanian, S., Habegger, L., Yu, H., Flicek, P., Clarke, L., Cunningham, F., Dunham, I., Zerbino, D., Zheng-Bradley, X., Lage, K., Berg Jespersen, J., Horn, H., Montgomery, S.B., DeGorter, M.K., Khurana, E., Tyler-Smith, C., Chen, Y., Colonna, V., Xue, Y., Gerstein, M.B., Balasubramanian, S., Fu, Y., Kim, D., Auton, A., Marcketta, A., Desalle, R., Narechania, A., Wilson Sayres, M.A., Garrison, E.P., Handsaker, R.E., Kashin, S., McCarroll, S.A., Rodriguez-Flores, J.L., Flicek, P., Clarke, L., Zheng-Bradley, X., Erlich, Y., Gymrek, M., Frederick Willems, T., Bustamante, C.D., Mendez, F.L., David Poznik, G., Underhill, P.A., Lee, C., Cerveira, E., Malhotra, A., Romanovitch, M., Zhang, C., Abecasis, G.R., Coin, L., Shao, H., Mittelman, D., Tyler-Smith, C., Ayub, Q., Banerjee, R., Cerezo, M., Chen, Y., Fitzgerald, T.W., Louzada, S., Massaia, A., McCarthy, S., Ritchie, G.R., Xue, Y., Yang, F., Gibbs, R.A., Kovar, C., Kalra, D., Hale, W., Muzny, D., Reid, J.G., Wang, J., Dan, X., Guo, X., Li, G., Li, Y., Ye, C., Zheng, X., Altshuler, D.M., Flicek, P., Clarke, L., Zheng-Bradley, X., Bentley, D.R., Cox, A., Humphray, S., Kahn, S., Sudbrak, R., Albrecht, M.W., Lienhard, M., Larson, D., Craig, D.W., Izatt, T., Kurdoglu, A.A., Sherry, S.T., Xiao, C., Haussler, D., Abecasis, G.R., McVean, G.A., Durbin, R.M., Balasubramaniam, S., Keane, T.M., McCarthy, S., Stalker, J., Chakravarti, A., Knoppers, B.M., Abecasis, G.R., Barnes, K.C., Beiswanger, C., Burchard, E.G., Bustamante, C.D., Cai, H., Cao, H., Durbin, R.M., Gerry, N.P., Gharani, N., Gibbs, R.A., Gignoux, C.R., Gravel, S., Henn, B., Jones, D., Jorde, L., Kaye, J.S., Keinan, A., Kent, A., Kerasidou, A., Li, Y., Mathias, R., McVean, G.A., Moreno-Estrada, A., Ossorio, P.N., Parker, M., Resch, A.M., Rotimi, C.N., Royal, C.D., Sandoval, K., Su, Y., Sudbrak, R., Tian, Z., Tishkoff, S., Toji, L.H., Tyler-Smith, C., Via, M., Wang, Y., Yang, H., Yang, L., Zhu, J., Bodmer, W., Bedoya, G., Ruiz-Linares, A., Cai, Z., Gao, Y., Chu, J., Peltonen, L., Garcia-Montero, A., Orfao, A., Dutil, J., Martinez-Cruzado, J.C., Oleksyk, T.K., Barnes, K.C., Mathias, R.A., Hennis, A., Watson, H., McKenzie, C., Qadri, F., LaRocque, R., Sabeti, P.C., Zhu, J., Deng, X., Sabeti, P.C., Asogun, D., Folarin, O., Happi, C., Omoniwa, O., Stremlau, M., Tariyal, R., Jallow, M., Sisay Joof, F., Corrah, T., Rockett, K., Kwiatkowski, D., Kooner, J., Tịnh Hiê`n, T., Dunstan, S.J., Thuy Hang, N., Fonnie, R., Garry, R., Kanneh, L., Moses, L., Sabeti, P.C., Schieffelin, J., Grant, D.S., Gallo, C., Poletti, G., Saleheen, D., Rasheed, A., Brooks, L.D., Felsenfeld, A.L., McEwen, J.E., Vaydylevich, Y., Green, E.D., Duncanson, A., Dunn, M., Schloss, J.A., Wang, J., Yang, H., Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Min Kang, H., Korbel, J.O., Marchini, J.L., McCarthy, S., McVean, G.A., Abecasis, G.R., 2015. A global reference for human genetic variation. Nature 526, 68–74.

Beignon, A.-S., McKenna, K., Skoberne, M., Manches, O., DaSilva, I., Kavanagh, D.G., Larsson, M., Gorelick, R.J., Lifson, J.D., Bhardwaj, N., 2005. Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor-viral RNA interactions. J. Clin. Invest. 115, 3265–3275.

Berghöfer, B., Frommer, T., Haley, G., Fink, L., Bein, G., Hackstein, H., 2006. TLR7 ligands induce higher IFN-alpha production in females. J. Immunol. 177, 2088–2096.

Bertoletti, A., Ferrari, C., 2016. Adaptive immunity in HBV infection. J. Hepatol. 64, S71–S83.

Boni, C., Fisicaro, P., Valdatta, C., Amadei, B., Di Vincenzo, P., Giuberti, T., Laccabue, D., Zerbini, A., Cavalli, A., Missale, G., Bertoletti, A., Ferrari, C., 2007. Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection. J. Virol. 81, 4215–4225.

Boni, C., Laccabue, D., Lampertico, P., Giuberti, T., Viganò, M., Schivazappa, S., Alfieri, A., Pesci, M., Gaeta, G.B., Brancaccio, G., Colombo, M., Missale, G., Ferrari, C., 2012.

Restored function of HBV-specific T cells after long-term effective therapy with nucleos(t)ide analogues. Gastroenterology 143, 963–973 e9.
Boonstra, A., Liu, B.-S., Groothuismink, Z.M., Bergmann, J.F., de Bruijne, J., Hotho, D.M., Hansen, B.E., van Vliet, A.A., van de Wetering de Rooij, J., Fletcher, S.P., Bauman, L.A., Rahimy, M., Appleman, J.R., Freddo, J.L., Reesink, H.W., de Knegt, R.J., LA Janssen, H., 2011. Potent immune activation in chronic hepatitis C patients upon administration of an oral inducer of endogenous interferons that acts via Toll-like receptor 7. Antivir. Ther. 17, 657–667.
Brouwer, W.P., Sonneveld, M.J., Tabak, F., Simon, K., Cakaloglu, Y., Akarca, U.S., Zeuzem, S., Ferenci, P., Heathcote, J.E., de Knegt, R.J., Boonstra, A., Hansen, B.E., Janssen, H.L., 2014. Polymorphisms of HLA-DP are associated with response to peginterferon in Caucasian patients with chronic hepatitis B. Aliment. Pharmacol. Ther. 40, 811–818.
Elgueta, R., Benson, M.J., de Vries, V.C., Wasiuk, A., Guo, Y., Noelle, R.J., 2009. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol. Rev. 229, 152–172.
Fakhir, F.-Z., Lkhider, M., Badre, W., Alaoui, R., Meurs, E.F., Pineau, P., Ezzikouri, S., Benjelloun, S., 2018. Genetic variations in Toll-like receptors 7 and 8 modulate natural hepatitis C outcomes and liver disease progression. Liver Int. 38, 432–442.
Ferrari, C., Penna, A., Bertoletti, A., Valli, A., Antoni, A.D., Giuberti, T., Cavalli, A., Petit, M.A., Fiaccadori, F., 1990. Cellular immune response to hepatitis B virus-encoded antigens in acute and chronic hepatitis B virus infection. J. Immunol. 145, 3442–3449.
Fischer, J., Weber, A.N.R., Böhm, S., Dickhöfer, S., El Maadidi, S., Deichsel, D., Knop, V., Klinker, H., Möller, B., Rasenack, J., Wang, L., Sharma, M., Hinrichsen, H., Spengler, U., Buggisch, P., Sarrazin, C., Pawlita, M., Waterboer, T., Wiese, M., Probst-Müller, E., Malinverni, R., Bochud, P.-Y., Gardiner, C., O'Farrelly, C., Berg, T., 2017. Sex-specific effects of TLR9 promoter variants on spontaneous clearance of HCV infection. Gut 66, 1829–1837.
Fonteneau, J.-F., Gilliet, M., Larsson, M., Dasilva, I., Münz, C., Liu, Y.-J., Bhardwaj, N., 2003. Activation of influenza virus-specific CD4+ and CD8+ T cells: a new role for plasmacytoid dendritic cells in adaptive immunity. Blood 101, 3520–3526.
Funk, E., Kottilil, S., Gilliam, B., Talwani, R., 2014. Tickling the TLR7 to cure viral hepatitis. J. Transl. Med. 12, 129.
Fuse, S., Tsai, C.-Y., Molloy, M.J., Allie, S.R., Zhang, W., Yagita, H., Usherwood, E.J., 2009. Recall responses by helpless memory CD8+ T cells are restricted by the up-regulation of PD-1. J. Immunol. 182, 4244–4254.
Gane, E.J., Lim, Y.-S., Gordon, S.C., Visvanathan, K., Sicard, E., Fedorak, R.N., Roberts, S., Massetto, B., Ye, Z., Pflanz, S., Garrison, K.L., Gaggar, A., Mani Subramanian, G., McHutchison, J.G., Kottilil, S., Freilich, B., Coffin, C.S., Cheng, W., Kim, Y.J., 2015. The oral toll-like receptor-7 agonist GS-9620 in patients with chronic hepatitis B virus infection. J. Hepatol. 63, 320–328.
Gibson, S.J., Lindh, J.M., Riter, T.R., Gleason, R.M., Rogers, L.M., Fuller, A.E., Oesterich, J.A.L., Gorden, K.B., Qiu, X., McKane, S.W., Noelle, R.J., Miller, R.L., Kedl, R.M., Fitzgerald-Bocarsly, P., Tomai, M.A., Vasilakos, J.P., 2002. Plasmacytoid dendritic cells produce cytokines and mature in response to the TLR7 agonists, imiquimod and resiquimod. Cell. Immunol. 218, 74–86.
Giltiay, N.V., Shu, G., Wang, T., Marken, J., Durcan, L., Clark, E.A., 2016. TLR7 responsiveness of human transitional B cells. J. Immunol. 196.
Griesbeck, M., Ziegler, S., Laffont, S., Smith, N., Chauveau, L., Tomezsko, P., Sharei, A., Kourjian, G., Porichis, F., Hart, M., Palmer, C.D., Sirignano, M., Beisel, C., Hildebrandt, H., Cénac, C., Villani, A.-C., Diefenbach, T.J., Le Gall, S., Schwartz, O., Herbeuval, J.-P., Autran, B., Guéry, J.-C., Chang, J.J., Altfeld, M., 2015. Sex differences in plasmacytoid dendritic cell levels of IRF5 drive higher IFN-α production in women. J. Immunol. 195, 5327–5336.
Holm, S., 1979. Board of the foundation of the Scandinavian journal of statistics a simple sequentially rejective multiple test procedure a simple sequentially rejective multiple test procedure. Source Scand. J. Stat. Scand J Stat. 6, 65–70.
Hou, J., Groothuismink, Z.M.A., Koning, L., Roomer, R., van IJcken, W.F.J., Kreefft, K., Liu, B.-S., Janssen, H.L.A., de Knegt, R.J., Boonstra, A., 2014. Analysis of the transcriptome and immune function of monocytes during IFNα-based therapy in chronic HCV revealed induction of TLR7 responsiveness. Antivir. Res. 109, 116–124.
Ida, J.A., Shrestha, N., Desai, S., Pahwa, S., Hanekom, W.A., Haslett, P.A.J., 2006. A whole blood assay to assess peripheral blood dendritic cell function in response to Toll-like receptor stimulation. J. Immunol. Meth. 310, 86–99.
Isogawa, M., Chung, J., Murata, Y., Kakimi, K., V Chisari, F., 2013. CD40 activation rescues antiviral $CD8^+$ T cells from PD-1-mediated exhaustion. PLoS Pathog. 9, e1003490.
Janssen, H.L.A., van Zonneveld, M., Senturk, H., Zeuzem, S., Akarca, U.S., Cakaloglu, Y., Simon, C., So, T.M., Gerken, G., de Man, R.A., Niesters, H.G., Zondervan, P., Hansen, B., Schalm, S.W., HBV 99-01 Study Group, and Rotterdam Foundation for Liver Research, 2005. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet 365, 123–129.
Janssen, H.L.A., Brunetto, M.R., Kim, Y.J., Ferrari, C., Massetto, B., Nguyen, A.-H., Joshi, A., Woo, J., Lau, A.H., Gaggar, A., Subramanian, G.M., Yoshida, E.M., Ahn, S.H., Tsai, N.C.S., Fung, S., Gane, E.J., 2017. Safety, efficacy and pharmacodynamics of vesatolimod (GS-9620) in virally-suppressed patients with chronic hepatitis B. J. Hepatol. 0.
Jego, G., Palucka, A.K., Blanck, J.-P., Chalouni, C., Pascual, V., Banchereau, J., Urbain, J., Leo, O., Moser, M., Thompson, J., Al, E., 2003. Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. Immunity 19, 225–234.
Jung, M.C., Spengler, U., Schraut, W., Hoffmann, R., Zachoval, R., Eisenburg, J., Eichenlaub, D., Riethmüller, G., Paumgartner, G., Ziegler-Heitbrock, H.W., 1991. Hepatitis B virus antigen-specific T-cell activation in patients with acute and chronic hepatitis B. J. Hepatol. 13, 310–317.
Krug, A., Towarowski, A., Britsch, S., Rothenfusser, S., Hornung, V., Bals, R., Giese, T., Engelmann, H., Endres, S., Krieg, A.M., Hartmann, G., 2001. Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. Eur. J. Immunol. 31, 3026–3037.
Lanford, R.E., Guerra, B., Chavez, D., Giavedoni, L., Hodara, V.L., Brasky, K.M., Fosdick, A., Frey, C.R., Zheng, J., Wolfgang, G., Halcomb, R.L., Tumas, D.B., 2013. GS-9620, an oral agonist of toll-like Receptor-7, induces prolonged suppression of hepatitis B virus in chronically infected chimpanzees. Gastroenterology 144, 1508–1517 e10.
Maan, R., van der Meer, A.J., Brouwer, W.P., Plompen, E.P.C., Sonneveld, M.J., Roomer, R., van der Eijk, A.A., Groothuismink, Z.M.A., Hansen, B.E., Veldt, B.J., Janssen, H.L.A., Boonstra, A., de Knegt, R.J., 2015. ITPA polymorphisms are associated with hematological side effects during antiviral therapy for chronic HCV infection. PLoS One 10 e0139317.
Martinet, J., Leroy, V., Dufeu-Duchesne, T., Larrat, S., Richard, M.-J., Zoulim, F., Plumas, J., Aspord, C., 2012a. Plasmacytoid dendritic cells induce efficient stimulation of antiviral immunity in the context of chronic hepatitis B virus infection. Hepatology 56, 1706–1718.
Martinet, J., Dufeu–Duchesne, T., Bruder Costa, J., Larrat, S., Marlu, A., Leroy, V., Plumas, J., Aspord, C., 2012b. Altered functions of plasmacytoid dendritic cells and reduced cytolytic activity of natural killer cells in patients with chronic HBV infection. Gastroenterology 143, 1586–1596 e8.
Mathan, T.S.M.M., Figdor, C.G., Buschow, S.I., 2013. Human plasmacytoid dendritic cells: from molecules to intercellular communication network. Front. Immunol. 4, 372.
Meier, A., Chang, J.J., Chan, E.S., Pollard, R.B., Sidhu, H.K., Kulkarni, S., Wen, T.F., Lindsay, R.J., Orellana, L., Mildvan, D., Bazner, S., Streeck, H., Alter, G., Lifson, J.D., Carrington, M., Bosch, R.J., Robbins, G.K., Altfeld, M., 2009. Sex differences in the Toll-like receptor–mediated response of plasmacytoid dendritic cells to HIV-1. Nat. Med. 15, 955–959.
Menne, S., Tumas, D.B., Liu, K.H., Thampi, L., AlDeghaither, D., Baldwin, B.H., Bellezza, C.A., Cote, P.J., Zheng, J., Halcomb, R., Fosdick, A., Fletcher, S.P., Daffis, S., Li, L., Yue, P., Wolfgang, G.H.I., Tennant, B.C., 2015. Sustained efficacy and seroconversion with the Toll-like receptor 7 agonist GS-9620 in the Woodchuck model of chronic hepatitis B. J. Hepatol. 62, 1237–1245.
Oh, D.-Y., Baumann, K., Hamouda, O., Eckert, J.K., Neumann, K., Kücherer, C., Bartmeyer, B., Poggensee, G., Oh, N., Pruss, A., Jessen, H., Schumann, R.R., 2009. A frequent functional toll-like receptor 7 polymorphism is associated with accelerated HIV-1 disease progression. AIDS 23, 297–307.
Rijckborst, V., ter Borg, M.J., Cakaloglu, Y., Ferenci, P., Tabak, F., Akdogan, M., Simon, K., Raptopoulou-Gigi, M., Örmeci, N., Zondervan, P.E., Verhey, E., van Vuuren, A.J., Hansen, B.E., LA Janssen, H., 2010. A randomized trial of peginterferon α-2a with or without ribavirin for HBeAg-negative chronic hepatitis B. Am. J. Gastroenterol. 105, 1762–1769.
Said, E.A., Al-Yafei, F., Zadjali, F., Hasson, S.S., Al-Balushi, M.S., Al-Mahruqi, S., Koh, C.Y., Al-Naamani, K., Al-Busaidi, J.Z., Idris, M.A., Balkhair, A., Al-Jabri, A.A., 2014. Association of single-nucleotide polymorphisms in TLR7 (Gln11Leu) and TLR9 (1635A/G) with a higher CD4T cell count during HIV infection. Immunol. Lett. 160, 58–64.
Savva, A., Roger, T., 2013. Targeting toll-like receptors: promising therapeutic strategies for the management of sepsis-associated pathology and infectious diseases. Front. Immunol. 4, 387.
Schott, E., Witt, H., Neumann, K., Taube, S., Oh, D.-Y., Schreier, E., Vierich, S., Puhl, G., Bergk, A., Halangk, J., Weich, V., Wiedenmann, B., Berg, T., 2007a. A Toll-like receptor 7 single nucleotide polymorphism protects from advanced inflammation and fibrosis in male patients with chronic HCV-infection. J. Hepatol. 47, 203–211.
Schott, E., Witt, H., Neumann, K., Bergk, A., Halangk, J., Weich, V., Müller, T., Puhl, G., Wiedenmann, B., Berg, T., 2007b. Association of TLR7 single nucleotide polymorphisms with chronic HCV-infection and response to interferon-a-based therapy. J. Viral Hepat. 42, 203–211.
Schreibelt, G., Tel, J., Sliepen, K.H., Benitez-Ribas, D., Figdor, C.G., Adema, G.J., de Vries, I.J., 2010. Toll-like receptor expression and function in human dendritic cell subsets: implications for dendritic cell-based anti-cancer immunotherapy. Cancer Immunol. Immunother. 59, 1573–1582.
See, P., Dutertre, C.-A., Chen, J., Günther, P., McGovern, N., Irac, S.E., Gunawan, M., Beyer, M., Händler, K., Duan, K., Bin Sumatoh, H.R., Ruffin, N., Jouve, M., Gea-Mallorquí, E., Hennekam, R.C.M., Lim, T., Yip, C.C., Wen, M., Malleret, B., Low, I., Shadan, N.B., Fen, C.F.S., Tay, A., Lum, J., Zolezzi, F., Larbi, A., Poidinger, M., Chan, J.K.Y., Chen, Q., Rénia, L., Haniffa, M., Benaroch, P., Schlitzer, A., Schultze, J.L., Newell, E.W., Ginhoux, F., 2017. Mapping the human DC lineage through the integration of high-dimensional techniques. Science (80-.) 356 eaag3009.
Seillet, C., Laffont, S., Trémollières, F., Rouquié, N., Ribot, C., Arnal, J.-F., Douin-Echinard, V., Gourdy, P., Guéry, J.-C., 2012. The TLR-mediated response of plasmacytoid dendritic cells is positively regulated by estradiol in vivo through cell-intrinsic estrogen receptor α signaling. Blood 119, 454–464.
Seillet, C., Rouquié, N., Foulon, E., Douin-Echinard, V., Krust, A., Chambon, P., Arnal, J.-F., Guéry, J.-C., Laffont, S., 2013. Estradiol promotes functional responses in inflammatory and steady-state dendritic cells through differential requirement for activation function-1 of estrogen receptor α. J. Immunol. 190, 5459–5470.
Souyris, M., Cenac, C., Azar, P., Daviaud, D., Canivet, A., Grunenwald, S., Pienkowski, C., Chaumeil, J., Mejía, J.E., Guéry, J.-C., 2018. *TLR7* escapes X chromosome inactivation in immune cells. Sci. Immunol. 3 eaap8855.
Swiecki, M., Gilfillan, S., Vermi, W., Wang, Y., Colonna, M., 2010. Plasmacytoid dendritic cell ablation impacts early interferon responses and antiviral NK and CD8+ T cell accrual. Immunity 33, 955–966.
Takahashi, K., Asabe, S., Wieland, S., Garaigorta, U., Gastaminza, P., Isogawa, M., Chisari, F.V., 2010. Plasmacytoid dendritic cells sense hepatitis C virus-infected cells, produce

interferon, and inhibit infection. Proc. Natl. Acad. Sci. 107, 7431–7436.
Tel, J., Schreibelt, G., Sittig, S.P., Mathan, T.S.M., Buschow, S.I., Cruz, L.J., Lambeck, A.J.A., Figdor, C.G., de Vries, I.J.M., 2013. Human plasmacytoid dendritic cells efficiently cross-present exogenous Ags to CD8+ T cells despite lower Ag uptake than myeloid dendritic cell subsets. Blood 121, 459–467.
van der Aa, E., van de Laar, L., Janssen, H.L., van Montfoort, N., Woltman, A.M., 2015. BDCA3 expression is associated with high IFN-lambda production by CD34(+)-derived dendritic cells generated in the presence of GM-CSF, IL-4, and/or TGF-beta. Eur. J. Immunol. 45, 1471–1481.
Villani, A.-C., Satija, R., Reynolds, G., Sarkizova, S., Shekhar, K., Fletcher, J., Griesbeck, M., Butler, A., Zheng, S., Lazo, S., Jardine, L., Dixon, D., Stephenson, E., Nilsson, E., Grundberg, I., McDonald, D., Filby, A., Li, W., De Jager, P.L., Rozenblatt-Rosen, O., Lane, A.A., Haniffa, M., Regev, A., Hacohen, N., 2017. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science (80-.) 356 eaah4573.
Woltman, A.M., den Brouw, M.L.O., Biesta, P.J., Shi, C.C., Janssen, H.L.A., 2011. Hepatitis b virus lacks immune activating capacity, but actively inhibits plasmacytoid dendritic cell function. PLoS One 6.