Kupffer Cells Interact With Hepatitis B Surface Antigen In Vivo and In Vitro, Leading to Proinflammatory Cytokine Production and Natural Killer Cell Function

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Background. Based on their localization, Kupffer cells (KCs) likely interact with hepatitis B virus (HBV). However, the role of KCs in inducing immunity toward HBV is poorly understood. Therefore, the interaction of hepatitis B surface antigen (HBsAg) and KCs, and possible functional consequences, were assessed.

Methods. KCs in liver tissue from patients with chronic HBV were analyzed for presence of HBsAg and their phenotype, and compared with KCs in control liver tissue. Liver graft perfusate–derived KCs and in vitro–generated monocyte-derived macrophages were investigated for functional interaction with patient-derived HBsAg.

Results. Intrahepatic KCs were HBsAg positive and more activated than those from control livers. KCs internalized HBsAg in vitro, which did not change their phenotype, but strongly induced proinflammatory cytokine production. Additionally, monocyte-derived macrophages also interacted with HBsAg, leading to activation and cytokine production. Furthermore, HBsAg-exposed macrophages and KC activated natural killer (NK) cells, resulting in increased CD69 expression and interferon-γ production.

Conclusions. KCs directly interact with HBsAg in vivo and in vitro. HBsAg-induced cytokine production by KCs and monocyte-derived macrophages and subsequent NK cell activation may be an early event in viral containment and may support induction of HBV-specific immunity upon HBV infection, but may also contribute to liver pathology.

Keywords. Kupffer cells; macrophages; HBV; HBsAg; innate immunity.

Hepatitis B virus (HBV) can cause chronic liver disease and may elicit progressive liver injury, leading to increased risk of developing liver cirrhosis, liver failure, and liver cancer [1, 2]. The immunological mechanisms determining the induction of effective antiviral immunity leading to self-limiting hepatitis or the lack of effective immune response toward the virus leading to chronic hepatitis B are still unclear. Although recently an HBV receptor has been identified on hepatocytes [3], the early steps in virus recognition by immune cells and the functional consequences of this interaction remain to be resolved.

Kupffer cells (KCs) are the resident macrophages of the liver [4, 5] that, besides being maintained by liver-derived precursors [6], have been shown to derive from monocytes [7] in a macrophage colony-stimulating factor (M-CSF)–dependent manner [5, 8]. They form, together with the sinusoidal endothelial cells, the first barrier for pathogens to enter the liver [9]. In humans, KCs have been identified by CD68 and CD14 expression

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Their number and location, but also typical macrophage properties such as endocytic capacity, expression of pattern recognition receptors, major histocompatibility complex (MHC) and costimulatory molecules, and ability to produce cytokines upon stimulation [10, 13–18], potentially render KCs effective immune cells contributing to viral clearance or persistence. Despite their potential, the exact role of KCs in HBV infection is still unclear. On one hand, KCs are considered to contribute to intrahepatic tolerance as demonstrated by rat KCs that hardly expressed proinflammatory cytokines, but preferably produced transforming growth factor–β upon exposure to HBV [19]. In contrast, stimulation of human nonparenchymal cells with HBV induced production of proinflammatory cytokines, which was ascribed to KC–hepatitis B surface antigen (HBsAg) interaction [20]. However, whether human KCs are able to bind/take up HBV is not known.

Therefore, we investigated the interaction between HBsAg and human KCs by assessing the presence of HBsAg within the CD14+ intrahepatic macrophage population and the phenotype of KCs during HBV infection as well as the functional consequences of HBsAg–KC interaction using both liver-derived KCs and in vitro–generated macrophages.

MATERIALS AND METHODS

KC Phenotype

Excess material of percutaneous needle liver biopsies obtained from 7 chronic HBV patients (Table 1) and 14 incisional biopsies obtained from donor liver during transplant was dissected, incubated in Roswell Park Memorial Institute medium (RPMI; Lonza) containing 0.5 mg/mL collagenase (Sigma-Aldrich) and 0.1 mg/mL DNAse (Roche), penicillin/streptomycin (Gibco), L-glutamine (Lonza), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Lonza) for 30 minutes, filtered through a 70-µm nylon cell strainer (BD) to acquire a single-cell suspension. Subsequently, cells were stained with antibodies against CD14+ cells.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age, y</th>
<th>Sex</th>
<th>HBV Genotype</th>
<th>Viral Load, gEq/mL</th>
<th>HBeAg Status</th>
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<tr>
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<tr>
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<td>7</td>
<td>27</td>
<td>F</td>
<td>B</td>
<td>2.86 × 10^7</td>
<td>+</td>
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</table>

Abbreviations: ALT, alanine aminotransferase; gEq, genome equivalent; HBeAg, hepatitis B early antigen; HBV, hepatitis B virus; ND, not determined.

HBsAg Staining on Patient-Derived Blood and Liver Tissue

Excess material of percutaneous needle liver biopsies were obtained from 13 chronic HBV patients and 9 non-HBV liver disease patients (Table 2), collected in RPMI and filtered through a 70-µm nylon cell strainer (BD) to acquire a single-cell suspension. In parallel, peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood of these patients by Ficoll-Paque (GE Healthcare) density gradient centrifugation. Liver cells and PBMCs were stained with antibodies against CD14 (MOP9) and CD45 (SK7; both BD) in PBS/1% fetal calf serum (FCS) (Sigma)/0.02% sodium azide, fixed with 2% formaldehyde, permeabilized with 0.5% saponin (VWR), and stained with an HBsAg-specific antibody (recognizing subtypes HBsAg Ad and Ay; Acris Antibodies GmbH). Data were acquired by flow cytometry and analyzed as described above.

The medical ethical committee of the Erasmus MC University Medical Center declared to have no objections against the use of excess patient biopsy material, and all liver disease patients gave informed consent before inclusion.

Cell Purification and Culture

HBV-negative healthy control liver mononuclear cells (LMNCs) were isolated from fresh liver graft perfusates derived from the second back table flush, which contains minimal contamination with donor peripheral blood cells, as previously described [21, 22]. LMNCs were isolated using Ficoll-Paque density gradient centrifugation and subsequently frozen for future use. KCs were isolated from these healthy control–derived LMNCs using CD14 magnetic-activated cell sorting (MACS) microbeads (Miltenyi Biotec) or fluorescence-activated cell sorting (FACS) by selecting CD14+ (M5E2; BD Pharmingen) cells using a FACSaria II (BD); purity ranged from 76% to 98% CD14+ cells.

To generate monocyte-derived macrophages, PBMCs were isolated from buffy coats from healthy blood donors (Sanquin) using Ficoll-Paque density gradient centrifugation. Monocytes were isolated using CD14 MACS microbeads. Macrophages were generated in 6-well plates (Costar) by culturing 1.5 × 10^6 monocytes in 2 mL/well of our standard culture medium (RPMI 1640 containing 10% heat-inactivated FCS, penicillin/streptomycin, L-glutamine, and HEPES, in the presence of 10 ng/mL M-CSF (R&D Systems). After 7 days, monocyte-derived macrophages were harvested and used in experiments.

Natural killer (NK) cells were isolated from healthy control–derived LMNCs or buffy coat–derived PBMCs using an NK
isolation kit (Miltenyi Biotec) or FACS by selecting CD3− (UCHT1) CD56+ (MY31; both BD) cells using a FACSAria II (BD).

**In Vitro Binding/Uptake of HBsAg**

In vitro–generated macrophages (5 × 10^4/well), fluorescence-activated cell-sorted KCs (5 × 10^4/well), or LMNCs (1 × 10^6/well) were cultured in 96-well plates in 125–150 µL standard culture medium, at 37°C in the absence or presence of 2.5 µg/mL patient plasma-derived HBsAg (pHBsAg) serotype Ay (American Research Products [ARP]) that was unlabeled or directly labeled via conjugation of DyLight 650 sulfhydryl-reactive dye (Thermo Fisher Scientific) to free reduced sulfhydryl groups, according to the manufacturer’s protocol. Unbound conjugate was removed by dialysis against PBS as dialysis buffer, using dialysis units of pore size 20 000 kDa (Thermo Fisher Scientific). After 1–4 hours, cells were harvested, fixed, and permeabilized as described above, and stained with biotin-labeled goat polyclonal anti-HBsAg Ad/Ay (Abcam) and streptavidin-peridinin chlorophyll (BD) or streptavidin-R-phycoerythrin (Invitrogen) or directly stained for surface marker CD14 (61D3) and CD3 (SK7; both eBioscience), and subsequently measured by flow cytometry and analyzed as described above. For confocal microscopy analysis, isolated KCs were incubated for 3 hours with medium or Dylight 650–conjugated pHBsAg at 37°C. KCs were then washed with PBS and fixed with 2% formaldehyde. During the fixation, KCs were transferred to 35-mm poly-D-lysine coated glass-bottom Petri dishes (MatTek Corporation) and spun down to adhere cells to glass bottom. After fixation, KCs were washed and stored in PBS/0.1% bovine serum albumin. KCs were analyzed on a Zeiss LSM 510 meta confocal microscope with helium-neon (633 nm) laser and 63 × oil-immersed objective. Digital magnification was 3×. Image J software was used to merge bright field and fluorescence images.

**HBsAg Stimulation of KCs and In Vitro–Generated Macrophages**

Liver perfusate–derived KCs (2 × 10^5/well) or in vitro–generated macrophages (2 × 10^5/well) were cultured in 96-well plates in 250-µL standard culture medium, in the absence or presence of 2.5 µg/mL pHBsAg (ARP) or Chinese hamster ovary cell-derived recombinant HBsAg (rHBsAg) (Prospec) for 48 hours. Supernatants were harvested, and cytokine production (interleukin [IL] 6, tumor necrosis factor [TNF], and

### Table 2. Patient Characteristics

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</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; gEq, genome equivalent; HBeAg, hepatitis B early antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; NA, not applicable; ND, not determined; PSC, primary sclerosing cholangitis.
IL-10) was determined by enzyme-linked immunosorbent assay (ELISA) (all kits from eBioscience). Cells were harvested, stained for CD40 (5C3; eBioscience), CD80 (MAB104; Beckman Coulter), and CD86 (IT2.2; Biolegend), measured by flow cytometry, and analyzed as described above.

**HBsAg Stimulation of LMNCs: Intracellular Cytokine Staining**

LMNCs (2.5 × 10⁶ cells/mL) were cultured in 96-well plates (Corning) in 250 µL X-VIVO culture medium (Lonza) containing penicillin/streptavidin (Gibco), L-glutamine (Lonza), and HEPES (Lonza) for 18 hours at 37°C with 5% carbon dioxide. Cells were either unstimulated or stimulated with 5 µg/mL pHBSAg (ARP). During the last 16 hours of culture, brefeldin A (10 µg/mL; Sigma-Aldrich) was added. Cells were harvested, incubated with LIVE/DEAD Fixable Aqua Dead Cell Stain (Invitrogen), fixed and permeabilized as described above, and stained for IL-6 (MQ2-13A5), TNF (Mab11; all eBioscience), CXCL8 | IL-8 (6217), IL-15 (34 559; both R&D Systems), IL-10 (JES3-19F1; BioLegend), IL-12p40 (C11.5) or CCL4 | MIP-1β (D21-1351; both BD Pharmingen), and CD14 (61D3) and CD45 (HI30; both eBioscience), measured by flow cytometry and analyzed as described above.

![Figure 1](image-url)

**Figure 1.** Kupffer cells (KCs) in chronically hepatitis B virus (HBV)-infected livers. KC phenotype (A) and activation status (B) were determined in liver biopsy samples from healthy controls (n = 11–14) and HBV patients (n = 5–7) by flow cytometry. Expression is shown as percentage positivity on CD14+ cells (A) or as mean fluorescence intensity (MFI) with background staining subtracted (B). CD14+ peripheral blood monocytes and KCs were analyzed for hepatitis B surface antigen (HBsAg) positivity in blood and liver biopsy samples of chronic hepatitis B patients (n = 13) and non-HBV-related liver disease patients (n = 9) by flow cytometry. Shown are representative dot plots (C) and individual and mean HBsAg positivity of all patients (D). *P < .05, **P < .01, ***P < .001, Mann–Whitney test. Abbreviation: HLA, human leukocyte antigen.
Figure 2. Hepatitis B surface antigen (HBsAg) interacts with healthy control–derived Kupffer cells (KCs) in vitro inducing cytokine production. Liver mononuclear cells (LMNCs) were incubated with/without fluorescent plasma-derived HBsAg (pHBsAg) (1 hour) and analyzed for HBsAg positivity in CD14− and CD14+ cells by flow cytometry. Shown are representative pseudo-color density plots (A) and HBsAg positivity in KCs (CD14) and T cells.
Macrophage–NK Cell Co-culture
A total of 1 × 10^6 liver perfusate-derived KCs or in vitro–generated macrophages was co-cultured with autologous perfusate-derived NK cells or peripheral blood NK cells, respectively, in a 1:1 ratio in 48-well plates in 250 µL standard culture medium with or without 2.5 µg/mL pHBSAg Ay (ARP). After 48 hours, supernatants and cells were harvested. Cells were stained with antibodies against CD3 (UCHT1), CD56 (MY31), and CD69 (L78; all BD), measured by flow cytometry and analyzed as described above. Supernatants were assessed for interferon gamma (IFN-γ) production by ELISA (eBioscience; detection limit 4 pg/mL).

RESULTS

KC Display an Activated Phenotype in Chronic HBV and Interact With HBsAg In Vivo
To investigate the effect of chronic HBV infection on KC, we compared the phenotype of CD45^+CD14^+ cells in liver biopsy tissue of chronic HBV patients with those in control liver tissue from healthy transplant donor livers (Supplementary Figure 1A–C). The percentages of KCs expressing CD11b, CD11c, CD163, and CD204 were comparable between both groups (Figure 1A), although the expression levels of CD11c and CD163 were significantly higher on KCs from HBV patients than on those from healthy controls (Supplementary Figure 1B; P < .05). Only the percentage of CD16-expressing KCs was significantly lower in chronic HBV patients than in healthy controls (Figure 1A). Furthermore, we observed that KCs from control liver tissues showed a low expression of CD40, which differed between donors, and were almost negative for CD80, whereas they expressed high levels of CD86 and MHC class I and II molecules (Figure 1B). The activation status of KCs in HBV-infected livers was found to be increased as demonstrated by significantly increased expression of CD40, HLA-ABC, and HLA-DR; also, the expression of CD86 tended to be increased (Figure 1B).

We next analyzed blood and liver biopsy samples from chronic HBV patients and patients with non-HBV-related liver disease for presence of HBsAg. Whereas a small, but significant, percentage of KCs from chronic HBV patients stained positive for HBsAg, peripheral blood CD14^+ monocytes from the same patients did not. As expected, CD14^+ cells derived from non-HBV patients were negative for HBsAg (Figure 1C and 1D). These data clearly indicate that KCs have the ability to interact with HBsAg in vivo and that CD45^+CD14^+ cells present in HBV-infected livers are significantly more activated than those present in healthy control livers.

HBsAg Interacts With KCs In Vitro, Inducing Cytokine Production
To further investigate this KC–HBsAg interaction, LMNCs or isolated KCs were incubated with pHBSAg in vitro. After incubation of LMNCs, very high HBsAg positivity was seen in CD14^+ KCs (90% ± 6.4%), whereas CD3^+CD56^+ T cells, used as a control population within the same LMNC population, showed hardly any HBsAg binding (Figure 2A and 2B). In addition, isolated KC became strongly positive for HBsAg, as demonstrated by flow cytometry using both detection of fluorescently labeled HBsAg and specific anti-HBsAg staining (Figure 2C). In parallel, confocal microscopy was performed to demonstrate that the interaction of KCs with HBsAg resulted in strong uptake (Figure 2D) by the large majority of cells. To investigate whether the activated phenotype of KCs in chronic HBV patients could be ascribed to a direct effect of HBsAg, liver graft perfusate–derived KCs were cultured in presence or absence of pHBSAg for 48 hours. These KCs expressed high levels of CD11b, intermediate to high levels of CD163 varying widely between donors (range, 18.5%–98.7% CD163^+ KCs), and low levels of CD204 (Supplementary Figure 2A). Expression of activation markers on perfusate-derived KCs was very similar to KCs present in healthy control liver tissue (Supplementary Figure 2B and data not shown). Exposure to neither pHBSAg nor rHBsAg changed the expression of costimulatory molecules (Figure 2E and data not shown), MHC molecules, or differentiation markers CD11b, CD163, and CD204 (data not shown).

To assess the effect of HBsAg on cytokine and chemokine production, LMNCs were cultured in the absence or presence of pHBSAg, and CD14^+ KCs were measured for intracellular cytokine expression. HBsAg induced many KCs to produce the proinflammatory cytokines IL-6 and TNF and chemokines CCL4 and CXCL8 in all donors tested, whereas it weakly stimulated IL-10 and IL-15 expression (Figure 2F). In addition, the percentage of IL-12p40–producing KCs was weakly enhanced in 4 of 5 experiments with different donors (Figure 2F). A time-course experiment with LMNCs and fluorescently labeled HBsAg showed that 1 hour of incubation with HBsAg was sufficient for strong HBsAg positivity in KCs (data not shown).
while inducing only a small percentage of TNF-producing KCs (Figure 2G). The percentage of TNF-producing KCs significantly increased upon 3 hours of HBsAg exposure and further increased upon 6 hours of exposure (Figure 2G). Similar results were obtained for IL-6 (data not shown). An exposure longer than 6 hours did not significantly increase the percentage of TNF-producing KCs, indicating that 6 hours is optimal to induce cytokine production. To confirm the direct effect of HBsAg on KCs, KCs were isolated from these perfusate-derived LNMCs and assessed for HBsAg-induced cytokine secretion. Both pHBsAg and rHBsAg stimulated KCs to secrete TNF, IL-10, and copious amounts of IL-6 (Figure 2H and Supplementary Figure 2C). Together, these data show that HBsAg is able to interact with KCs, leading to HBsAg internalization and induction of proinflammatory cytokine production without inducing an activated phenotype based on costimulatory molecule and MHC expression.

HBsAg Also Functionally Interacts With In Vitro–Generated Macrophages

KCs are thought to originate from liver precursor cells as well as from monocytes in an M-CSF–dependent manner [5, 7, 8]. Because monocyte-derived macrophage development likely increasingly occurs during inflammation, a situation often present in HBV-infected livers, we also investigated the effect on M-CSF–driven human monocyte–derived macrophages. Compared to perfusate-derived KCs, in vitro–generated macrophages expressed similar levels of CD11b, CD40, CD80, and CD86, and lower levels of CD163, CD204, and CD86, and lower HLA-ABC (Supplementary Figure 2A and Supplementary Figure 3A–D). Upon pHBsAg exposure, in vitro–generated macrophages interacted actively with HBsAg as demonstrated by a high percentage of HBsAg–positive cells (Figure 3A). In contrast to KCs, incubation of in vitro–generated macrophages with pHBsAg induced an activated phenotype showing increased CD40 and CD80 expression, and decreased CD86 expression (Figure 3B and Supplementary Figure 3E), besides inducing IL-6, TNF, and IL-10 production (Figure 3C).

HBsAg-Activated Macrophages Induce NK Cell Function

To further examine the functional consequence of the interaction between HBsAg and macrophages, we investigated the interaction of KCs and in vitro–generated macrophages with autologous liver-derived NK cells and peripheral blood-derived NK cells, respectively. When culturing in vitro–generated macrophages with NK cells in the presence of pHBsAg, NK cells not only strongly upregulated CD69 expression (Figure 4A), but also significantly increased IFN-γ production (Figure 4B). The basal CD69 expression of liver NK cells is already high, as demonstrated before [23], and could hardly be further increased upon exposure to HBsAg-stimulated KCs, making it difficult to assess this marker. Also for the KC–NK cell co-cultures, induction of IFN-γ production by NK cells was demonstrated (Figure 4C). Adding pHBsAg to NK cells, KCs, or macrophages alone neither changed CD69 expression nor induced IFN-γ production (data not shown).

DISCUSSION

A virus-induced innate immune response is generally considered to be crucial for early viral containment and the induction of virus-specific immunity. However, due to delayed onset of symptoms in HBV-infected humans and lack of appropriate animal models, HBV-induced innate immune responses are largely undefined. We demonstrated, for the first time, a direct interaction between HBsAg and KC both in vivo and in vitro. The interaction of KC with HBsAg resulted in HBsAg uptake, induction of cytokine production, and the induction of IFN-γ production by NK cells. Similar findings were observed for in vitro–generated monocye-derived macrophages.

Whether the HBsAg acquired by intrahepatic KCs is the result of a direct interaction between HBsAg as part of the whole virion, subviral particle, or the uptake of HBV/HBsAg-containing hepatocytes remains to be elucidated. Neither HBsAg positivity of KCs nor activation status of KCs significantly correlated with serum HBV DNA levels. The enhanced expression of CD80 and HLA-DR tended to be lower on KCs present in hepatitis B early antigen (HBeAg)-infected patients compared with HBeAg-uninfected patients (data not shown); however, this was not observed for the other activation markers. Whether this possible reduction in activation status in HBeAg+ patients corresponds to an immune regulatory effect of HBeAg, as suggested before [17, 24–26], will be subject of future research. So far, our patient group was too small, and patient numbers should be largely increased to make proper subgroups and perform statistical analysis to discriminate between effects of differential alanine aminotransferase levels, HBV load, HBsAg levels, and HBeAg expression. The relatively low percentage and staining intensity of HBsAg+ KCs in HBV-infected livers compared to the signals obtained from KC–HBsAg interaction in vitro seems counterintuitive, but could have several explanations. First, HBsAg+ KC detection by anti-HBsAg antibody instead of directly fluorescent HBsAg used in vitro was significantly lower (data not shown). Additionally, it is tempting to speculate that the longer exposure time of KCs to HBsAg in vivo may not further increase the level of antigen uptake, but rather impairs HBsAg detection as it is known that HBsAg, once taken up, will be broken down by macrophages [27]. Furthermore, the higher activation status of KCs present in these chronically infected livers may negatively influence the uptake capacity of macrophages as has been shown for dendritic cells, cells closely related to macrophages [28, 29].

The receptors involved in KC–HBsAg interaction will be the subject of future research, but several candidate receptors exist.
CD14 has been demonstrated as an interesting candidate receptor on monocytes. Recently, HBsAg positivity was demonstrated in CD14⁺ monocytes of chronic HBV patients, using a very sensitive Tissuefax system [30]. Due to a possibly less sensitive detection method as discussed above, we cannot exclude the presence of HBsAg⁺ monocytes, and therefore the role of CD14 in HBsAg binding cannot be ruled out. Another receptor possibly involved in HBsAg–KC interaction not expressed by monocytes is the macrophage mannose receptor, known to be involved in HBsAg interaction with dendritic cells [31]. Altogether, it seems plausible to conclude that KCs contain higher levels of HBsAg than circulating monocytes, which could be explained by cell-intrinsic properties of macrophages and/or higher levels of HBV/HBsAg in the intrahepatic compartment.

The absence of HBsAg-induced upregulation of costimulatory and MHC molecules on KCs in vitro suggests that indirect activation underlies the activated phenotype of KCs in HBV-infected livers. Assessing activation of KCs in liver biopsies from a non-HBV liver disease control group could be informative to discern between an indirect or direct effect of the virus. However, the large heterogeneity of such a control group will make it difficult to interpret these data. In addition, the scarcity of such liver material is a limiting factor. Furthermore, because HBsAg exposure of in vitro–generated monocyte-derived macrophages induced an activated phenotype, an alternative explanation could be that the activated phenotype observed is the result of activated infiltrating monocyte-derived macrophages, as an increased differentiation of monocytes into macrophages.
is likely to occur during inflammatory responses [32–34]. Because monocytes, monocyte-derived macrophages, and KCs share many surface markers, it is hard to discern between these cell populations.

Although in vitro HBsAg exposure did not affect KC phenotype, it induced KCs to secrete high levels of proinflammatory cytokines. HBsAg-induced production of IL-6, TNF, and CXCL8 by KCs is comparable with the HBV-induced production of these cytokines by nonparenchymal liver cells, presumably KCs, reported by Hösel et al [20]. These cytokines are possibly beneficial for the early viral containment, as cytokines such as IL-6 and TNF directly inhibit HBV replication [20, 31], and could provide the first signals toward the initiation of HBV-specific immunity in the early phase after HBV infection by attracting and activating other immune cells. The IFN-γ production by NK cells upon co-culture with HBsAg-activated macrophages is likely mediated by KC-derived cytokines such as IL-12 and IL-15 [10]. Interestingly, also in woodchuck hepatitis virus, intrahepatic IFN-γ and IL-12 expression were found in the early phase after infection, and also the HBV chimpanzee model demonstrated intrahepatic IFN-γ levels before clear onset of the adaptive T-cell response [35, 36]. Although we cannot rule out a contribution of T-cell-derived IFN-γ in these models, elevated NK cell frequencies have been reported in patients with acute HBV [37]. Together with KC-derived cytokines, these NK cell–derived factors may further control viral replication and may favor appropriate dendritic cell maturation required for T helper 1 cell priming, supporting induction of virus-specific immunity [38]. In addition, although KCs were recently described to restrict liver damage [39], these cytokines could also be harmful, contributing to liver inflammation and liver fibrosis [40, 41].

In conclusion, the present study shows a direct interaction between KCs and HBsAg, in vivo and in vitro. It is tempting to speculate that HBsAg-induced KC function and subsequent NK cell activation, as demonstrated here, contribute to inhibition of HBV replication and induction of HBV-specific immunity during the early phase after HBV infection, and/or to liver damage during the chronic phase, but further research is required to investigate these hypotheses.

**Supplementary Data**

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of
data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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