Compromised Function of Natural Killer Cells in Acute and Chronic Viral Hepatitis

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Background. Natural killer (NK) cells are an integral part of the innate immune system. They have been suggested to play an important role in both defense against viral hepatitis and the pathogenesis of other liver diseases.

Methods. NK cells from 134 individuals including patients with acute hepatitis B and C as well as chronic hepatitis B, C, and delta (D) patients were studied.

Results. Infection with viral hepatitis was associated with increased frequencies of NK cells in the peripheral blood; that NK cells showed a less activated phenotype and were compromised in cytolytic function and cytokine production in all viral hepatitis infections: Hepatitis virus infections did not alter NK cell differentiation, and the activity and severity of liver disease were reflected by alterations of NK cell surface receptors as demonstrated by principal component analysis.

Conclusion. NK cell phenotypic and functional alterations can equally be observed in HBV, HCV, and HDV infections. Instead, patterns of NK cell alterations differ in acute and chronic infections. Thus, our data suggest a common mechanism in the alteration of NK cell phenotype and function with unique variations that depend on disease activity rather than virus-specific factors.

Keywords. NK cells; HBV; HCV; HDV; viral hepatitis.
into CD56brightCD16+/− and CD56dimCD16+ NK cells [16]. The potent effector functions of NK cells are tightly regulated via numerous activation and inhibitory receptors [17].

Previous studies regarding phenotype and function of NK cells in patients with viral hepatitis have revealed, in part, conflicting results. NK cells have exhibited an activated phenotype in some studies [18, 19], an effect that has been associated with both spontaneous recovery, from acute hepatitis C infection [20] but also attenuated responses [21]. While the inhibiting NK cell receptor, KIR2DL3, is associated with higher chances of spontaneous clearance but not chronicity [22], the opposite is observed for an activating receptor, KIR2DS3 [23]. In chronic hepatitis B and C, a suppressed functionality of NK cells have been observed that potentially contributes to viral persistence [24, 25]. Yet other studies suggest increased cytolytic responses of NK cells and implicate a role for these cells in the pathogenesis of HBV and HCV infection [25–27]. The presence of certain inhibitory NK cell receptors have been associated with the response to antiviral treatment in hepatitis C [28], and treatment-induced decline of HBV viral load have been shown to restore NK cell function [29]. The question if hepatitis viruses interact directly with NK cells and shape their responses has not been answered conclusively. HCV may bind NK cells via HCV-E2-CD81 interaction [30, 31], but functional consequences of this interaction are still discussed [32, 33]. Finally, NK cells have recently been suggested to regulate HBV-specific adaptive immunity by killing of HBV-specific CD8 T cells [34]. Overall, it is evident that there is an alteration of NK cells in hepatitis virus infections though a comprehensive picture of NK cell phenotype and function in acute and chronic infections is still missing. Previous studies have mainly investigated the expression of single surface molecules, while no comprehensive investigation of a broader repertoire of inhibitory and activating receptors has been performed. Finally, it has been unclear if observed changes in NK cell phenotype are due to distinct effects of the underlying hepatitis virus or due to an altered inflammatory environment. To date there is yet to be a study that compares both acute and chronic viral hepatitis, which also incorporates different hepatitis viruses. Thus, the aim of this study was to perform a comprehensive investigation of NK cell phenotype and function in large cohorts of well-characterized patients with acute and chronic viral hepatitis B, C, or chronic hepatitis delta.

**MATERIAL AND METHODS**

**Patient Material**

We included 134 individuals in this study. Patients with acute and chronic viral hepatitis were seen in the outpatient clinic of the Department of Gastroenterology, Hepatology, and Endocrinology at Hannover Medical School in Germany. Patients gave informed consent for the investigation of immunological parameters as part of different protocols that had been approved by the ethics committee of the Hannover Medical School, Hannover, Germany. Individual clinical study protocols have been described in detail previously [35–38], and only patients with compensated liver disease have been selected. Patient cohorts and characteristics are described in Table 1. None of the patients received interferon (IFN)-based antiviral therapy at the time of the present investigation, and all tested negative for human immunodeficiency virus (HIV) infection. All patients with acute HBV infection cleared the infection spontaneously, and acute HCV patients who did not clear spontaneously received IFN-based antiviral therapy after 12 weeks. Healthy donors were recruited at the Karolinska University Hospital, Stockholm, Sweden after approval by the regional ethics committee. PBMCs were isolated through standard density-gradient separation and cryopreserved from all study subjects for later analysis. All experiments were performed at the Centre of infectious medicine (CIM) at the Karolinska University Hospital, Stockholm, Sweden. To avoid any bias control, experiments comparing healthy controls from Hannover and Stockholm have been performed. No differences in subset distribution or expression of surface markers could be found, with the exception of DNAM-1 (data not shown).

**Phenotypical Stainings**

Frozen PBMCs were thawed and washed twice with phosphate-buffered saline (PBS) followed by a staining for 30 minutes in the dark at room temperature (RT) with saturating amounts of the desired monoclonal antibody (mAb) combinations. Subsequently, PBMCs were washed once and incubated for 20 minutes in the dark at RT with streptavidin-conjugates. Finally, cells were washed twice and fixed in 1% paraformaldehyde (PFA) for 10 minutes in the dark at 4°C and then washed once before acquisition on the flow cytometer. The monoclonal antibodies (mAbs) and the exact combinations used for the phenotypical stainings are listed in the supplementary online information. The data were acquired on a BD LSRFortessa cell analyzer and analyzed with FlowJo software version 9.5 (Tree-star Inc, Ashland, OR).

**Functional NK Cell Assays**

Frozen PBMCs were thawed and washed twice with PBS and afterward rested overnight or stimulated with 100 ng/mL IFN-α and cocultured with K562 target cells as described elsewhere [19, 39]. The detailed protocol is available in the Supplementary material.

**Statistics**

Data were analyzed using GraphPad Prism (GraphPad Software, La Jolla, CA). The test used in each graph is stated in the respective figure legends. In general P values of <.05 were considered to be significant. Principal component analysis (PCA)
Table 1. Patient Cohorts and Clinical Characteristics of Patients With Complete Data Available Used in Subsequent Statistical Analysis

<table>
<thead>
<tr>
<th>Parameter/Cohort</th>
<th>Healthy</th>
<th>Acute HCV</th>
<th>Chronic HCV</th>
<th>Acute HBV</th>
<th>Chronic HBV</th>
<th>Chronic HDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>32</td>
<td>11±</td>
<td>17±</td>
<td>10±</td>
<td>17±±</td>
<td>31±±</td>
</tr>
<tr>
<td>Sex</td>
<td>50% male (16F/16M)</td>
<td>45% male (8F/5M)</td>
<td>59% male (7F/10M)</td>
<td>50% male (5F/5M)</td>
<td>41% male (10F/7M)</td>
<td>54% male (14F/17M)</td>
</tr>
<tr>
<td>Age</td>
<td>48 (21–65)</td>
<td>36 (21–58)</td>
<td>47 (30–75)</td>
<td>43 (20–69)</td>
<td>41 (22–59)</td>
<td>41 (20–61)</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>na</td>
<td>101 (43–887)</td>
<td>49 (21–139)</td>
<td>868 (47–5295)</td>
<td>30 (18–62)</td>
<td>59 (24–339)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>na</td>
<td>425 (130–1155)</td>
<td>80 (17–180)</td>
<td>1828 (28–4176)</td>
<td>30 (14–166)</td>
<td>76 (23–360)</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>na</td>
<td>32 (11–123)</td>
<td>12 (4–56)</td>
<td>183 (7–423)</td>
<td>84 (82–116)</td>
<td>79 (64–110)</td>
</tr>
<tr>
<td>Prothrombin (% of normal)</td>
<td>na</td>
<td>98 (89–128)</td>
<td>96 (40–109)</td>
<td>58 (33–83)</td>
<td>95 (82–116)</td>
<td>79 (64–110)</td>
</tr>
<tr>
<td>gGT (U/L)</td>
<td>na</td>
<td>163 (12–507)</td>
<td>52 (7–642)</td>
<td>103 (36–391)</td>
<td>22 (6–120)</td>
<td>45 (15–460)</td>
</tr>
<tr>
<td>Platelets (Thd/µL)</td>
<td>na</td>
<td>253 (173–389)</td>
<td>205 (134–887)</td>
<td>210 (165–451)</td>
<td>236 (118–369)</td>
<td>144 (88–331)</td>
</tr>
<tr>
<td>HDV RNA quant (copy/mL)</td>
<td>na</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>HBV DNA (IU/mL)</td>
<td>na</td>
<td>nd</td>
<td>nd</td>
<td>53 900 (131–310 000)</td>
<td>2945 (341–330 000)</td>
<td>14 35 (22–279 000)</td>
</tr>
<tr>
<td>HbsAG (IU/mL)</td>
<td>na</td>
<td>nd</td>
<td>nd</td>
<td>2185 (1078–22 484)</td>
<td>2190 (96–22 371)</td>
<td>10 746 (1088–38 072)</td>
</tr>
<tr>
<td>HCV RNA (IU/mL)</td>
<td>na</td>
<td>1500 (890–415 000)</td>
<td>1 225 000 (86 600–8 500 000)</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
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All data are shown as median with range.
Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis delta virus; na, not available; nd, not detected.

a Only patients with complete datasets are shown in the table.

b 13 out of 17 HBV-infected patients are anti-HBe+.

RESULTS

NK Cells Exhibit a Less Activated Phenotype in Viral Hepatitis

The foregoing findings prompted us to analyze in detail the NK cell populations and a shift in the NK cells compartment toward a more CD56bright phenotype in infections with hepatitis viruses. Increased frequencies of NK cells were associated with higher expression of TRAIL on CD56bright NK cells when compared to those with chronic hepatitis virus infections. In general, NK cells from patients with acute and chronic hepatitis infections showed increased frequencies of CD56bright NK cells compared to healthy controls (Figure 1A). The frequency of chronic HBV and HDV infection increased in patients with chronic hepatitis infections compared to healthy controls (Table 1, Figure 1B). The frequency of chronic HBV and HDV infection increased in all hepatitis infections with the exception of the group with chronic HCV infection compared to healthy controls (Figure 1C). CD56dim NK cells were significantly higher in all hepatitis infections with the exception of acute HCV infections compared to healthy controls (Figure 1D). CD56dim NK cells were significantly higher in chronic HCV or HDV infection but not in individuals with chronic HBV. Expression of the activating receptor CD244 was lower in HDV-infected patients compared to chronic HBV and chronic HDV infections. The observed increases in the overall frequency of CD56bright NK cells were similarly altered in frequency among patients with chronic hepatitis infections and healthy controls (Figure 1E). The frequencies of circulating NK cells from patients with different forms of acute and chronic hepatitis virus infections were associated with increased frequencies of CD56bright NK cells. The frequencies of circulating NK cells from patients with different forms of acute and chronic hepatitis virus infections were associated with increased frequencies of CD56bright NK cells. The frequencies of circulating NK cells from patients with different forms of acute and chronic hepatitis virus infections were associated with increased frequencies of CD56bright NK cells.
Figure 1. Hepatitis virus infections lead to increased levels of NK cells. A, Representative staining of NK cells from one individual showing the gating strategy used to identify total, CD56\textsuperscript{dim}, and CD56\textsuperscript{bright} NK cells. B–D, Frequencies of total (B), CD56\textsuperscript{dim} (C), and CD56\textsuperscript{bright} (D) NK cells. E, Distribution of CD56\textsuperscript{dim} and CD56\textsuperscript{bright} subsets depicted as frequency of total NK cells. The graph represents counts of healthy controls (n = 30) and patients with acute HBV (n = 12), chronic HBV (n = 22), acute HCV (n = 10), chronic HCV (n = 17) and chronic HDV (n = 27). *P < .05; **P < .01; ***P < .001, Mann–Whitney test; horizontal bars represent mean. Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis delta virus; NK, natural killer.
Figure 2. Phenotypic characteristics of NK cells in hepatitis virus infections. Summary of the expression (MFI) of CD244, CD25, CD48, CD161, DNAM-1 and TRAIL on total, CD56dim, and CD56bright NK cells in healthy controls (n = 30); patients with acute HBV (n = 11) and HCV (n = 9), chronic HBV (n = 20) and HCV (n = 15) or HDV (n = 26). *P < .05; **P < .01; ***P < .001, Mann–Whitney test; horizontal bars represent mean. Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis delta virus; MFI, mean fluorescence intensity; NK, natural killer.
decreased expression of activation-associated receptors, with some differences between acute and chronic infection. However, the patterns for HBV and HCV were similar, suggesting a mechanism for alteration of NK cell phenotypes more depending on disease activity rather than on virus-specific factors.

**NK Cell Maturation Is not Influenced by Different or Multiple Hepatitis Virus Infections**

Our observation of increased CD56\textsuperscript{bright} NK-cell frequencies in viral hepatitis suggests an altered NK-cell maturation in these patients. CD56\textsuperscript{bright} NK cells are considered to be predecessors of CD56\textsuperscript{dim} NK cells [40]. Moreover, within the CD56\textsuperscript{dim} compartment, stages of differentiation can be determined by staining for the expression of CD57, NKG2A, and KIRs [40]. Previous work has shown that viral infection can alter the differentiation status of CD56\textsuperscript{dim} NK cells [41]. Taking our lead from this finding, we analyzed expression of NKG2A, pan-KIR, and CD57 on CD56\textsuperscript{dim} NK cells from our patient cohort (Figure 3A). Neither CD57 (Figure 3B) nor total KIR expression (Figure 3C) differed between the patient groups. For HCV, the frequency of NKG2A-positive cells was significantly higher in patients with an acute infection compared to chronic infection (Figure 3D), the same trend was observed in acute HBV infection compared to chronic HBV infection. Boolean analysis of differentiation within the CD56\textsuperscript{dim} NK cell subset did not reveal any additional differences (Supplementary Figures 1A and B). Thus, hepatitis virus infections do not influence the differentiation status of NK cells, and no differences were found.
when comparing acute and chronic hepatitis infections, with the exception of NKG2A that was slightly higher in the acute phase of hepatitis infections.

Functional Responses Are Blunted During Infection With Hepatitis Viruses

To analyze functional responses in the different patient cohorts we assessed degranulation, cytokine, and chemokine production against K562 cells, with or without prior IFN-α stimulation (Figure 4A). Resting NK cells responded with similar levels of CD107α and IFN-γ against K562 cells in healthy donors and patients (Figure 4B and 4C), whereas MIP-1β production was markedly lower in patients vs controls (Figure 4D). This reduction was especially pronounced for acute HBV as compared to patients with other hepatitis infections. Tumor necrosis factor α (TNF-α) production showed a similar trend among the patients (Figure 4E). After IFN-α stimulation NK cells responded with higher levels of degranulation, as well as cytokine and chemokine production. But NK cells from patients, independent of acute or chronic infection, expressed a lower inducibility of functional responses by IFN-α compared to the healthy individuals (Figure 4A–D). Except for lower macrophage inflammatory protein 1 (MIP-1) β-production in the acute HBV patients, no other major differences in function were detected when comparing the different patient cohorts. Finally, we analyzed multifunctional responses (Figure 4F). Multifunctional NK cells expressing 3–4 functions simultaneously were much more common in the healthy controls (Figure 4F). Despite a slight trend toward an overall lower functionality in acute patients as compared to chronic patients, no significant differences were detected in multifunctionality when comparing the different infections (Figure 4F). In summary, NK cells from all patient cohorts exhibited blunted functional responses No differences in degranulation or cytokine production were evident between the different hepatitis viruses, except in the aforementioned cases.

Principal Component Analysis Identifies Distinct Phenotypic and Functional Patterns Distinguishing Different Forms of Viral Hepatitis

The detailed analysis of the phenotypic and functional properties of NK cells derived from patients with acute and chronic HBV, HCV, as well as HDV presented above included a total of 49 unique NK cell parameters. Although at some instances significant differences emerged between the phases of disease, or the different viruses, it became clear that no single parameter was enough to sufficiently separate the infections and/or phases of disease from each other. Therefore, as a next step, we performed a principal component analysis (PCA) with these 49 entities and an additional 10 clinical parameters to further improve the separation of individuals with acute vs the respective chronic infections, and of patients with milder and more advanced disease activity (Figure 5).

Using this method, NK cells from hepatitis patients could clearly be separated from NK cells from healthy controls. This distinction was driven by the expression of CD244 and CD48 as well as by cytokine production after co-culture with target cells (Figure 5A, left panel). Moreover, patients with acute viral hepatitis could be separated from individuals with chronic infections in terms of NKG2A, TRAIL, CD48, and CD161 expression, a higher frequency of CD56bright NK cells, as well as MIP-1β production (Figure 5B, left panel). Investigating the differences between the acute and chronic phases of HBV and HCV infections, separately, showed that similar phenotypic and functional markers could differentiate acute and chronic hepatitis for both infections (Figure 5C and 5D, left panels). PCA also revealed that patients with acute hepatitis B and C differed in NKG2A expression (Figure 5E, left panel), whereas no distinct differences in NK cell properties between patients with chronic hepatitis B and chronic hepatitis C were detected (Figure 5F, left panel). Furthermore, differences in disease activity may explain the observed separation between chronic hepatitis B and hepatitis D patients (Figure 5G, left panel).

Similar NK cell parameters differentiating the patient groups were obtained irrespective of the inclusion of clinical parameters. Not surprisingly, clinical parameters indicating biochemical activity of liver disease or liver function were identified as the most powerful markers to differentiate acute vs chronic infections and chronic HBV vs HDV infection (Figure 5A–G, right panel).

Overall, PCA revealed distinct NK cell characteristics for acute and chronic viral hepatitis and clearly separated virus-infected individuals from healthy controls. However, we did only detect minor differences in between the 3 viruses.

DISCUSSION

This comprehensive analysis of NK cell phenotype and function in viral hepatitis has revealed distinct features of NK cells in HBV, HCV, and HDV infections. Although various previous studies have described alterations of single surface molecules in either acute vs chronic infections or in virus-infected individuals vs healthy controls [42, 43], no study has yet incorporated the different infections in their various phases as controls. Overall, our data suggest a reshaping of the NK cell pool during hepatitis infections toward more CD56bright NK cells, and altered functional capacity resulting in impaired cytolytic activity and reduced cytokine production. Importantly, and in contrast to some previous studies, NK cell phenotypic and functional alterations were equally observed in HBV, HCV, and HDV infections, and thus, the suggested functional dichotomy between chronic hepatitis B and C virus-infected patients [25] was not confirmed. Although that study also found decreased

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Figure 4. Decreased functional responses of NK cells during infection with hepatitis viruses. Frequency of CD107a⁺ (A), IFN-γ⁺ (B), MIP-1β⁺ (C) and TNF⁺ (D) total NK cells summarized for all groups. E, Multifunctional NK cell responses grouped after amount of function. Multifunctional subsets are identified using a Boolean-analysis, based on the single positive subsets. Total pie size depicts the total percentage of NK cells responding of a theoretical maximum of 100% depicted by the empty circle around the healthy controls. Counts of individuals in the graphs for healthy controls (n = 25), acute HBV (n = 10), chronic HBV (n = 14), acute HCV (n = 9), chronic HCV (n = 14) and chronic HDV (n = 13). *P<.05; **P<.01; ***P<.001, Mann–Whitney test; horizontal bars represent mean. Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis delta virus; IFN, interferon; MIP-1, macrophage inflammatory protein 1; NK, natural killer; TNF, tumor necrosis factor.
Figure 5. Principal component analysis (PCA) of NK cell phenotype and function in viral hepatitis. PCA plots for the patients and control cohorts, taking into consideration FACS derived parameters (left plot) or FACS derived parameters and clinical data (right plot). Both panels only show the PCA plot based on the parameters that differ significantly ($P < .05$) between the different groups. The 6 most significant parameters are shown on the side of the PCA plots.
cytokine production by NK cells from patients with chronic hepatitis B and C, they reported higher cytotoxicity against murine target cells, spontaneous and redirected, but not against K562 cells. The observed differences in cytotoxicity could be explained by a different methodology to investigate effector functions.

The observed higher expression of NKG2A on NK cells in acute hepatitis B relative to acute hepatitis C patients may be a sign of disease activity, rather than a unique feature of HBV infection. NKG2A was indeed one of the most robust markers in separating patients with acute vs chronic hepatitis indicating that this molecule may serve as a NK cell activation marker in hepatitis virus infection. This finding fits with previous reports indicating that NKG2A expression on NKT cells is associated with spontaneous clearance of acute hepatitis C [20]. Lower expression of both CD244 and CD48 in the patients would indicate that NK cells are less likely to be activated via this route. The higher frequency of CD161 on NK cells from patients with chronic hepatitis C is in line with earlier findings [44] showing that patients with acute hepatitis C who progress toward the chronic stage had higher levels of CD161 on the surface of their NK cells. Additionally, and in line with our previous findings [19], TRAIL expression on NK cells was able to distinguish samples from patients with acute hepatitis from chronic hepatitis. Thus, alterations in NK cell phenotype and function seem to depend more on disease activity rather than on factors specific for a single virus.

The most recent work examining the role of NK cells in hepatitis delta, published in the 1980s, suggests some associations between NK cell activation and viral replication or response to IFN-α treatment [45–47]. It is interesting to note that in our present study, NK cell phenotype and function were not associated with HDV viral loads (data not shown). However, we found a higher frequency of total and CD56dim NK cells in hepatitis delta patients, indicating altered NK cell maturation. NK cells from HDV-infected patients produced the highest amounts of IFN-γ and TNF-α among the different hepatitis virus infections. Principal component analysis confirmed that activation markers and functional response differentiated hepatitis delta patients from chronic HBV monoinfected individuals. This finding could in part explain the faster progression toward liver damage in hepatitis delta patients compared to other hepatitis virus infections. Independent of this, given the disease severity of hepatitis delta, clearly, more in-depth studies are warranted.

A key finding of this study was that NK cells showed a marked impairment of function and an overall inhibited phenotype in all groups of viral hepatitis. Functional impairment was broad, covering degranulation as well as cytokine and chemokine production. Patients studied here were still viremic at the time of investigation, and thus, we may speculate that this functional impairment contributed to the lack of control of viral hepatitis as previously suggested [48]. In addition, NK cells may contribute to the prevention of fibrosis progression by killing hepatic stellate cells, which play a key role in the production of collagen in the liver [49]. Thus, dysfunctional NK cells could lead to a more rapid fibrosis progression.

We observed a restructuring of the NK cell compartment with a relative increase of NK cells in viral hepatitis as compared to healthy controls. This increase was most pronounced in the CD56bright NK cell subset and could hint toward changes in the maturation status of NK cells in hepatitis B and C. This observed increase in CD56bright NK cells could help to explain, in part, the lower functional responses, because CD56bright NK cells respond less vigorously. Previous findings indicate that viral infections can influence the maturation status of NK cells [40, 41]. Interestingly, however, differentiation within the CD56dim NK cell subset was not affected in any of the hepatitis virus infections, and this is in line with what has been described during chronic recurrent herpes simplex virus type 2 infection in humans [50].

Our findings argue against a direct effect specific to any single hepatitis virus on NK cells and plays down the importance of previous studies suggesting altered NK effector functions following exposure to HCV virions [33]. Indeed, a more recent study could not confirm altered effector functions of NK cells exposed to HCV infectious virions [32]. Even though we did not study functional consequences of exposure of NK cells to virions in vitro, our observations do not support a major direct viral effect on NK cells as phenotypes. and functions were rather similar between patients infected with HBV, HCV, and HDV. Furthermore, no correlations between NK cell phenotype and function with HBV, HCV, or HDV viral load could be found in our dataset (data not shown). However, preliminary data of our laboratory showed rather normal phenotypes and function of NK cells in patients with nonviral inflammatory fatty liver disease (data not shown). Thus, even though no differences between the different hepatitis viruses were evident, infections per se seem to modulate NK cells.

In summary, NK cell frequencies are increased in viral hepatitis, and NK cells express an inhibited phenotype with blunted functional responses against target cells. There were no striking

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Figure 5 continued. plots. P values are derived from either a t-test when comparing 2 groups or an ANOVA when comparing multiple groups. A, Results from all healthy controls and all patients grouped according to disease status. B, Comparison of acute vs chronic infection. C–G, Comparison of acute vs chronic infection with the indicated viruses. Counts of individuals in the graphs for healthy controls (n = 30), acute HBV (n = 10), chronic HBV (n = 17), acute HCV (n = 11), chronic HCV (n = 17) and chronic HDV (n = 25). For the significance calculation in the PCA model t-test and ANOVA are used. Abbreviations: ANOVA, analysis of variance; FACS, fluorescence-activated cell sorter; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis delta virus; NK, natural killer.

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differences in NK cell phenotype and function between the different hepatitis viruses. However, NK cells were differentially altered in acute and chronic hepatitis. We suggest that disease activity but not the specific virus, per se, drives NK cell activation and functionality.

**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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