Editorial

JOURNAL OF HEPATOLOGY

HCMV jogs the 'memory' of NK cells in HBV

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The treatment paradigm in hepatitis B virus (HBV) is rapidly transforming with many new viral and immune therapies entering the clinical trial pipeline. Although many of the immune targets focus on restoration of the exhausted virus-specific T cell response[1] an increasing body of work makes a case for natural killer (NK) cell-based immune therapies and vaccines.[2] Advances in NK cell biology have revealed that these 'innate' cells may also possess adaptive features. These cells 'sitting-on-the-fence' of innate-adaptive immunity, undergo significant changes in their repertoire during differentiation. A repeating stimulus such as cytomegalovirus (CMV) infection may result in the accumulation of highly differentiated NK cells, designated as 'memory-like'. These 'adaptive' NK cells rapidly expand and generate an effective 'recall' response. Features of memory-like NK cells have not been widely reported in chronic hepatotropic infections (HBV and HCV) where co-infection with human CMV (HCMV) is usually universal.[3] In this issue, Schuch, Zecher *et al.*, describe for the first time the expansion of memory-like NK cells in patients with chronic HBV infection in conjunction with HCMV.[4]

In the setting of HCMV, which has a broad impact on immune responses and induces an adaptive reconfiguration of the NK cell compartment; NK cell subsets predominantly express the terminal differentiation marker CD57, important in the control of HCMV. These cells also express the transmembrane protein DAP-12 coupled with NKG2C, a receptor of the C-type lectin family and the inhibitory CD85j.[5] This distinct memory-NK cell subset has a variable expression of cell surface receptors, reduced expression of signalling proteins and transcription factors compared to conventional NK cells. Epigenetic modifications are also known to drive the emergence and persistence of HCMV-adapted memory-NK cells (Fig. 1).[6] There is now an increased focus on studying 'antigen'-specific (memory) NK cell responses in humans, previously neglected despite their description in mice. In HIV memory-NK cells have been shown to demonstrate increased antibody-dependent cell cytotoxicity (ADCC) and a potential reduced capacity for T cell regulation, unlike conventional NK cells.[7,8]

In HBV infection, previous reports have described the expansion of functional NK cells following exposure to therapeutic pegylated interferon alpha (Peg-IFNα) with or without nucleos(t)ide analogue therapy.[9,10] This expansion is however of proliferating functional CD56^{bright} NK cells, mediated via IL-15. This is of importance as the liver is enriched with CD56^{bright} NK cells, and in a study where mice lacked T and B cells, CXCR6-postive liver NK cells were found to mediate hapten-specific hypersensitivity.[11] Prolonged expansion of NK cells with antigen-specific responses is disparate to the traditional view where NK cells are considered short-lived populations with rapid turnover and contraction following acute responses. However, it is now accepted that NK cells are diverse, possess both innate- and adaptive-like properties and even memory-like-NK cell subsets may have differences in receptor, intracellular molecule and transcription factor expression (Fig. 1). The *in vitro* exposure of NK cells to the cytokine combination of IL-12/15/18 has shown the potential to generate memory-like cells with increased effector functions.[12] The signalling pro-inflammatory cytokines appear to be pivotal to the long-term maintenance of NK cells with memory-like responses; IL-12/15 being especially important for CMV-specific, cytokine-induced and liver-resident memory-NK cells. NK cell memory may be antigen-specific or antigen-independent. Antigen-independent memory-like NK cells may expand following exposure to specific cytokines which can imprint long-lasting changes on their effector functions.[13]

Schuch, Zecher *et al.*, confirm that HCMV infection is common in HBV-infected patients and report the expansion of FcεRIγ− CD56^{dim} NK cells in HBV compared to healthy donors.[4] It has been reported that NK cells deficient for the adaptor protein FcεRIγ convincingly associate with HCMV.[14] In this article, FcεRIγ− CD56^{dim} NK cells have been designated as 'memory-like' NK cells as they share many of the characteristics of adaptive NK cells. The Fc chain associates with the transmembrane portion of CD16 to form the low-affinity IgG, FcγRIII also expressed on NK cells and thus they respond to CD16 stimulation and akin to NKG2C+ NK cells mount robust responses against HCMV-infected targets.[15] In line with this, the authors

Keywords: Antibody-dependent cell cytotoxicity (ADCC); Cytomegalovirus (CMV); Epigenetics; FcεRIγ; Hepatitis B virus (HBV); Memory-NK cell; Metabolism.
Received 13 November 2018; accepted 15 November 2018
☆ DOI of original article: http://dx.doi.org/10.1016/j.jhep.2018.10.006.
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show augmented responses of memory-like FcεRIγ− CD56^{dim} NK cells to CD16 stimulation.[4] The expression of signalling molecules EAT2, SyK and transcriptional regulators PLZF and Helios were analysed. Together with the downregulation of CD7, the expression of EAT2, SyK, PLZF and Helios was also reduced on FcεRIγ− CD56^{dim} memory-NK cells, with the noted expansion of CD57 in keeping with previous reports.[15]

NK cell receptors exhibit varying expression in HBV. The activatory C-type lectin NKG2C was upregulated on memory-like FcεRIγ− CD56^{dim} NK cells, with a compensatory downregulation of the inhibitory NKG2A.[10] NKG2C and NKG2A are often mutually exclusive and thus the overexpression of NKG2C can shift the response to the non-classical major histocompatibility complex class I molecule, HLA-E from inhibitory, as seen with a predominance of NKG2A+/NKG2C− cells to an activating phenotype; NKG2C+/NKG2A-. Schuch, Zecher *et al.*, also report reduced expression of the natural cytotoxicity receptors (NCRs) NKp30 and NKp46 along with Siglec-7 on memory-like FcεRIγ− CD56^{dim} NK cells, however the expression of these markers was also markedly different on bulk CD56^{dim} NK cells in HBV-infected patients compared with healthy donors. Interestingly, the expression of NKG2A, NKG2C, NKp30, NKp46, Siglec-7 and CD2 on the bulk CD56^{dim} NK cell population correlated with their frequency on memory-like FcεRIγ− CD56^{dim} NK cells.[4] CD2 has been identified as a key co-stimulatory receptor, which like the Fc receptors, contributes to increased cytokine production in adaptive NK cells after synergising with CD16.[16]

NK cells have been shown to expand and proliferate in response to IL-15, which is also described for memory-NK cells with features of 'recall' capacity.[12] The memory-like FcεRIγ− CD56^{dim} NK cells investigated here display a resting phenotype with the reduced proliferative capacity of FcεRIγ− CD56^{dim} NK cells *vs.* FcεRIγ+ CD56^{dim} NK cells, as previously described for FcεRIγ deficient NK cells,[14] especially in healthy donors, as HBV-infected patients did retain Ki67+ CD56^{dim} NK cells. This once again, highlights the diverse phenotype of adaptive/memory-NK cells. BCL2 analysis showed significant upregulation on FcεRIγ− CD56^{dim} NK cells compared with the FcεRIγ+ subset.[4] Similarities in global epigenetic modifications have been described between adaptive NK cells and memory CD8 T cells[6] which drive the alteration in metabolic pathways and impact functional properties of NK cells.[17] Glucose uptake was reduced, yet polarised and functional mitochondria were increased on memory-NK cells; a pre-requisite for mitochondrial oxidative phosphorylation (OXPHOS). Interestingly, while memory-like and conventional NK cells differed in their metabolic potential, this was not influenced by HBV infection.[4] In contrast, metabolic requirements of CD8 T cells in HBV demonstrate mitochondrial dysfunction; exhausted antigen-specific T cells are dependent on abundant glucose supplies, unlike functional CMV-specific T cells which can utilise OXPHOS in the absence of glucose.[18]

Importantly memory-like FcεRIγ− CD56^{dim} NK cells in HBV demonstrate a CD16-dependent increase in degranulation with increased IFNγ production. Intriguingly in response to stimulation with cytokine cocktails IL-12/15/18, IFNγ production was diminished from memory-like FcεRIγ− CD56^{dim} NK cells (Fig. 1). Certain transcription factors, such as PLZF interact with several target genes, including IL12RBw, IL18RAP and KLRB1, which may account for the lack of responsiveness to cytokine stimulation.[6] DNA methylation is another feature of adaptive NK cells, silencing PLZF, SyK and EAT2 in line with the report by Schuch, Zecher *et al.* Investigating DNA methylation of memory-like FcεRIγ− CD56^{dim} NK cells, Schuch, Zecher *et al.*, analysed the expression of FcεRIγ and Helios on NK cells. FcεRIγ+/Helios+ and FcεRIγ-/Helios+ make up the largest and smallest subsets of bulk CD56^{dim} NK cells respectively, but importantly CD16-induced degranulation was increased on the FcεRIγ-/Helios- subset, compared to conventional FcεRIγ+/Helios+ NK cells. Hypermethylation of the FCERIG promoter in CD56^{dim} NK cells lacking FcεRIγ and CpG hypomethylation of the IFNG CNS1 and IFNG promoter region in FcεRIγ-/Helios- cells was demonstrated, confirming that FcεRIγ and Helios expression phenotypically, functionally and epigenetically regulate memory-like CD56^{dim} NK cells in HBV.[4]

Schuch, Zecher *et al.*, in HBV, have prompted an avenue of previously neglected research into memory-NK cells in relation to HCMV. This is critical for a number of reasons and not least as the liver is a major site of HCMV infection[19] and the expansion

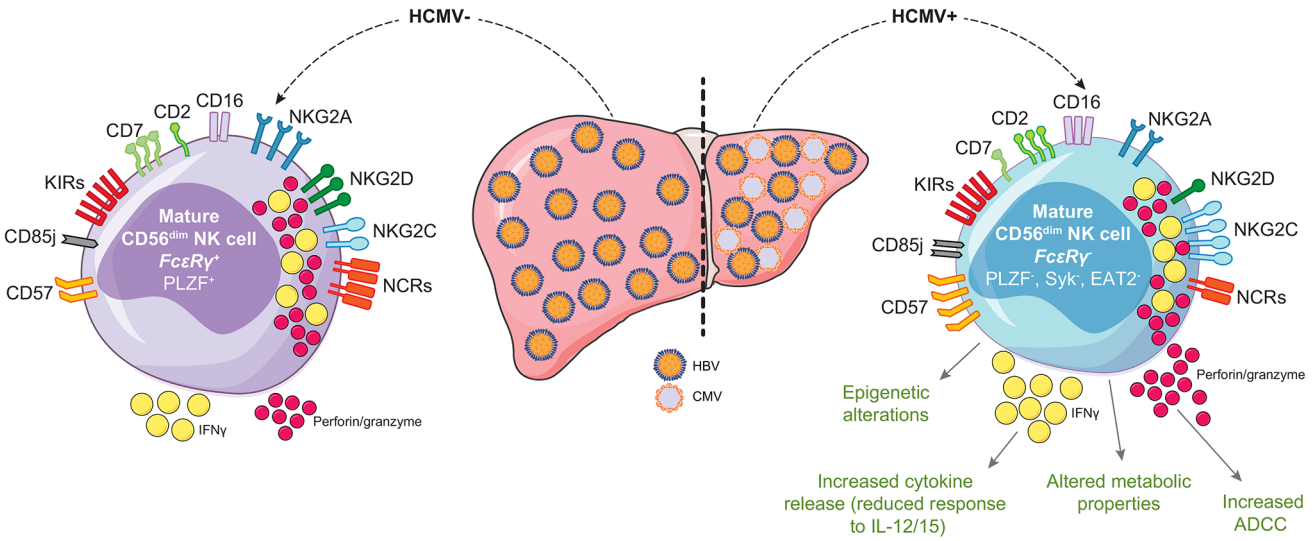

Fig. 1. Differences in FcεRIγ+ and FcεRIγ− CD56^{dim} mature NK cells in HBV. FcεRIγ− CD56^{dim} NK cells are depicted as memory-like NK cells with differential receptor expression, a diverse transcription profile along with epigenetic and metabolic alterations compared to conventional FcεRIγ+ CD56^{dim} NK cells, following exposure to HCMV. In addition, memory-like NK cells undergo increased cytokine responses and CD16-mediated ADCC. ADCC, antibody-dependent cell cytotoxicity; HBV, hepatitis B virus; HCMV, human cytomegalovirus; IFN, interferon; NCR, natural cytotoxicity receptor; NK, natural killer.

of adaptive NK cells may serve to combat HCMV; whether this is also true for hepatotropic infections remains to be seen. As noted, in this article liver tissue-specific memory-like NK cells were not analysed and thus tissue-related HCMV activations cannot be excluded, but should be a line of further investigation along with the analysis of transcriptionally distinct tissue-resident NK cells.[20] In addition, the impact of age, gender and ethnicity on these cells in HBV is required along with the exploration of inhibitory markers such as PD-1.[21] ADCC persists in FcεRIγ− NK cells in HIV despite viral load reduction with long-term anti-retroviral therapy,[22] whether such similarities are seen in HBV requires further investigation, along with the modulation of these cells with novel agents. Finally, ADCC has importance for the design of NK cell therapy/vaccine type trials to promote HBs antibody responses and thus the further study of memory-NK cells in HBV is urgently required.

Financial support

USG was supported by a Wellcome Trust Clinical Research Training Fellowship (107389/Z/15/Z) and an NIHR Academic Clinical Lectureship. LG-M received funding from National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (RO1DK106491).

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Acknowledgements

The authors would like to thank Dr Dimitra Peppa (University of Oxford) for intellectual discussions and input.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2018.11.009.

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