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CD4 T cells in hepatitis B virus: “you don’t have to be cytotoxic to work here and help”.

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EDITORIAL TITLE: ***CD4 T cells in hepatitis B virus: "you don't have to be cytotoxic to work here and help".***

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Understanding the immunopathogenesis of chronic hepatitis B (CHB) in full will be key to delivering the hepatitis B virus (HBV)-cure program, allowing us to induce a range of host-leukocyte responses that act in concert to achieve complete viral elimination. While previous research has understandably focused on restoring the function of exhausted virus-specific CD8 T cells, there has been a relative lack of appreciation for the role played by the CD4 T cell pool and virus-specific CD4+ clones in controlling HBV infection [1]. It is well established that CD4 T cells exert a multitude of critical functions in host immunity, including promotion of B cell antibody production, driving granulocyte recruitment to sites of infection and supporting activation of antigen presenting cell (APC) populations via CD40:CD40L interactions [2]. Of particular relevance in the context of CHB is the ability of naïve CD4 T cells to differentiate into functionally distinct subsets under the control of specific transcription factors and polarising cytokines. Major subtypes include T-helper (Th)1 cells which produce IFNγ/IL-12, Th2 mainly producing IL-4, Th17 producing IL-17/IL-21 (dependent upon RORγT), and the T-regulatory (T-reg) population which depends on FoxP3. Despite the recent focus on CD8 T cell function in HBV research, data from

the 1980's has previously indicated that CD4 T cells are the dominant supporters of CD8 T cell-mediated HBV responses and play essential roles in driving CD8 T cell effector function and memory formation [3]. Indeed, lack of CD4 T cells was even considered to be a major cause of exhaustion among virus-specific CD8 T cells, which are dependent upon a continued supply of multiple cytokines to maintain their effector functions [4]. In line with this concept, other investigators have also observed that loss of CD4 T cells during acute infection can generate a defective memory CD8 T cell compartment, including the loss of IL-2 production, thus mounting only weak responses upon secondary pathogen exposure [5]. While chronic viral infections clearly have potential to induce exhaustion in CD4 T cell populations similarly to their CD8 T cell counterparts, this area is notably understudied at present. A recent report indicated that during chronic infection, CD4 T cells display increased expression of mRNAs encoding transcription factors implicated in the development of different Th cell subsets. These data indicate that exhausted CD4 T cells do not merely diminish in their ability to produce cytokines - they also exhibit an altered functional profile as a consequence of change in differentiation patterns [6]. In particular, CD4 T cells have been shown to act as potent producers of IL-21 which likely sustains CD8 T cell responses to chronic infections, hence differentiation towards this functional profile may be an important determinant of patient outcome. In the absence of IL-21 derived from CD4 T cells, virus-specific CD8 T cells appear to lose their ability to produce IL-2, TNFα and IFNγ and display other hallmarks of an exhausted state (including high levels of CD43 and PD-1) [7]. Consistent with this finding, a recent study in CHB patients showed that combined OX40 stimulation and PD-L1 blockade significantly augmented IFNγ and IL-21 producing HBV-specific CD4 T cells *in vitro*, suggesting that such immunotherapeutic approaches could potentially also improve virus-specific CD4 T cells responses *in vivo* [8].

Seroclearance of chronic viral infection is thought to be mediated primarily by IFNγ release, but the CD4 T cell pool may simultaneously exacerbate immunopathology via production of TNFα which potentially worsens hepatic injury (as previously described in a mouse model of concanavalin-A induced hepatitis [9]). Indeed, a recent study identified that IL-17 producing CD4 T cells (Th17) enriched in the blood and liver of CHB patients are closely associated with hepatic flares, during which they display marked expression of the pro-inflammatory cytokines IL-1β, IL-6, TNFα [10]. Multiple other studies also suggest that the CD4 T cell compartment may represent an important 'inflammatory' component of CHB pathology, including an increased expression of NKG2D ligands which blunt HBV-specific CD4 T cell responses with their interaction of the NKG2D receptor on NK cells [11]. Whether operating in a regulatory or pro-inflammatory role, it is clear that CD4 T cells have a major part to play in mediating viral clearance and control, but until recently studies in CHB have been lacking.

In this edition of *Journal of Hepatology,* Wang *et al.*, perform a thorough immunological analysis of the HBV-specific CD4 T cell response in patients in various disease phases of CHB. They report that distinct subsets of HBV-specific CD4 T cells producing TNFα or IFNγ are associated with liver damage or viral clearance, respectively. The authors utilised a panel of HLA-restricted epitopes against CD4 T cells and undertook analysis with these HBV peptides against the core (HBc) and surface (HBs) (envelope) proteins [12]. A previous study has described

that increasing CD4 T cell responses to HBc and HBeAg occur during seroconversion to anti-HBe and anti-HBs production in acute HBV infection, implying that T cells directed against these proteins are essential for viral elimination. In contrast, patients with CHB failed to mount an efficient T cell response to HBc/HBs, thereby contributing to persistence of infection [13]. Wang *et al.*, report a significant overall expansion of TNFα-producing, compared to IFNγ-expressing, HBc- and HBs-specific CD4 T cells in their patient cohort. Accounting for patient clinical parameters, the authors report a dominance of TNFα-producing HBV-specific CD4 T cells in HBeAg+ patients with high viral load, whereas IFNγ-expressing HBV-specific CD4 T cells instead dominated in patients who approached viral clearance (HBeAg seroconversion and HBsAg loss). Importantly HBsAg level negatively correlated with the dominance of IFNγ+ HBV-surface specific CD4 T cells. Thus viral clearance is accompanied by an elevation in both the frequency and dominance of antigen-specific CD4 T cells producing IFNγ, which has also been observed for other viral infections such as CMV [14] and bacterial infections [15].

It is now widely accepted that induction of hepatic flares in CHB may in fact aid seroclearance (HBsAg loss), such that discontinuation of nucleos(t)ide analogue (NA) therapy has now been described as a potential therapeutic strategy in a number of different studies [16]. In order to circumvent potential for excessive hepatic flares and possible liver failure, studies of treatment discontinuation are undertaken in a controlled manner and thus large flares are carefully avoided. Coinciding with this, virus-specific CD4 T cell responses in patients stopping NA therapy with a mild-moderate rise in transaminases demonstrate only limited virus-specific CD4 T cell responses [17, 18]. It is notable therefore that Wang *et al.*, studied patients presenting with severe hepatic flares prior to commencing NA therapy. Consequently, they observed potent TNFα+ (core>surface) virus-specific CD4 T cell responses in patients with aggressive liver damage, followed by significant decreases in these responses only after NA introduction. In contrast, IFNγ+ virus-specific CD4 T cell responses in these patients were characteristic of individuals capable of clearing HBsAg [12]. These findings are consistent with previous reports of high circulating serum TNFα in patients with active hepatitis B [19], indicating that this cytokine may contribute to liver damage and immunopathology (presumably with a view to gaining viral control and allowing for subsequent induction of virus-specific T cells expressing IFNγ). Of note, TNFα/IFNγ double producers and IFNγ+ single producers were routinely detected in patients undergoing HBeAg and HBsAg seroconversion, suggesting that antigen specific-CD4 T cell responses transition from TNFα to IFNγ expression in individuals undergoing viral clearance *(Figure 1).* Both populations were observed to express the lineage-specific transcription factors, T-bet and RORγT at comparable levels, suggesting that Th1 and Th17 pathways of differentiation may be important in this context (although IL-21 rather than IL-17 appeared critical in this case). Further work will now be required to elucidate this process in full and identify the transcription factor profile associated with mono-production of TNFα.

The importance of T cell differentiation in shaping the cytokine responses that switch patients from an inflammatory profile (TNFα dominated) to achieving viral clearance (IFNγ) has been underappreciated in the context of CHB pathology and therapy. The authors have shed new light on the profile of CD4 T cells in CHB and

're'-opened important avenues for the future study of these crucial cells. This work should encourage other researchers in the field to now consider the influence of CD4 T cell differentiation pathways and downstream interactions with CD8 T, B and innate leukocyte populations on anti-viral responses. Such analyses should not only be undertaken in the blood but also within the intrahepatic compartment, both in treatment-naïve subjects and those undergoing therapy via longitudinal sampling [20]. Ultimately, therapeutic approaches that can increase T-bet expression and enhance IL-21 synthesis in patient CD4 T cells may succeed in boosting virus-specific CD8 T cell responses and augmenting strategies for HBV cure.

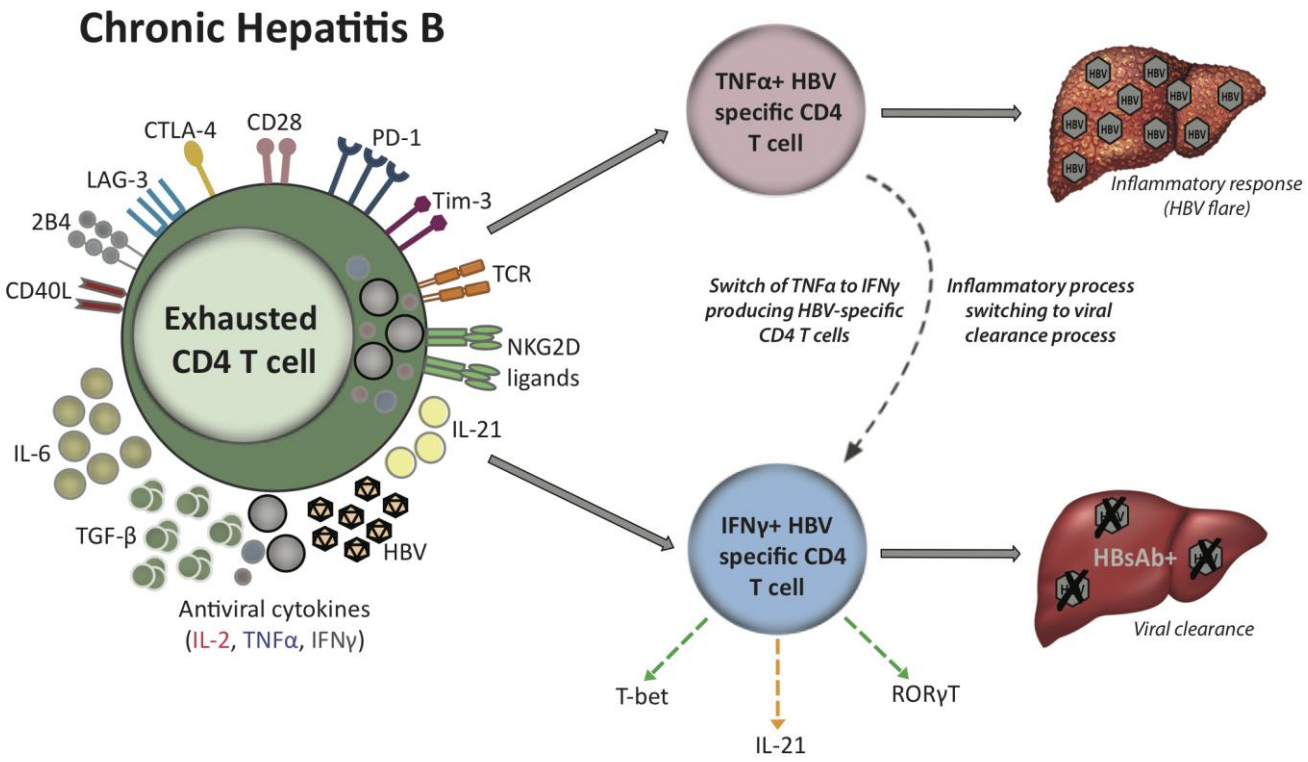

Figure 1: Diagram depicting the phenotype of an exhausted CD4 T cell in chronic hepatitis B infection. The expression of TNFα/IFNγ producing HBV-specific CD4 T cells during HBV infection is represented and how this leads to the generation of an inflammatory response or that of viral clearance respectively. A switching of cytokine producing from TNFα to IFNγ producing virus specific CD4 T cells may progress towards viral clearance, with the expression of other cytokines and transcriptions factors governing the differentiation process.

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