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Pegylated Interferon-α for chronic hepatitis B…not ready to be shelved yet! New insights on its role using Single-Cell Transcriptomics.

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Abstract:

Pegylated-Interferon alpha (PegIFN-α) is a treatment option for chronic hepatitis B (CHB) but has limited efficacy. It has both immune modulatory and antiviral capacity, however the mechanisms of PegIFN-α in relation to CHB pathogenesis remain understudied. The current editorial reviews a recent study related to the *in-vivo* use of PegIFN-α using single-cell RNA sequencing (scRNA-seq) technology. PegIFN-α reverts the transcriptome profile of treated samples close to that of the healthy population, diminishing the pro-inflammatory genes and 'inflammatory scores' which will be important for the HBV-cure program.

Functional cure for chronic hepatitis B (CHB) has now become an expectation amongst clinicians and scientists, with pharmaceutical companies being invested in the development and delivery of novel viral and immune targets. Whilst new agents remain under investigation, nucleos(t)ide analogue (NA) and Pegylated-Interferon alpha (PegIFN-α) remain the mainstay of treatment, with the use of these in various combinations globally. Understanding the immunopathogenesis of hepatitis B virus (HBV) is critical to delivering HBV-cure, allowing us to induce a range of host-leukocyte responses that act in concert to achieve complete functional cure. Moreover, an appreciation of host immune and viral interactions during therapy in CHB is underscored by the limited studies, especially with regards to the impact of therapy in the intrahepatic environment. The application of innovative technologies, such as single-cell RNA sequencing (scRNA-seq) with transcriptomic analysis can provide important information on the immune interactions involved in disease pathogenesis. Hou et al., studied mRNA transcripts extracted from paraffin embedded tissue sections from liver biopsies of patients from various disease phases in CHB, delineating important alterations which are induced locally in chronic infection (1). A recent study delineated a comprehensive landscape of immune cells across the disease spectrum of CHB, showing that exhausted CD8+ T cells were preferentially expanded in immune active patients and that these cells exhibited significant interactions with CD4+ T cells and FCGR3A+ macrophages. They also demonstrated populations of dysregulated MAIT, T-regs and myeloid subsets in cohorts of patients with 'benign disease' in treatment naïve patients and identified a circulating CXCR3+ GNLY+ CD8+ central memory T cell population which might serve as a surrogate for predicting HBV clearance (2). Further studies have analysed mRNA transcripts determining metabolic changes in HBV and also in the ability to predict treatment cessation (3), however there are limited data on the immune transcriptome in patients undergoing treatment with HBV therapies.

Antiviral therapy for CHB has largely satisfied an unmet need of providing viral suppression using NAs, which may reduce the complications of CHB, but have little impact on HBsAg levels. The effect of NAs on T cell responses has shown a partial recovery of CD4 and CD8 T cell function. The functional recovery of antiviral immunity is likely to be dependent on the ability of NAs to reduce liver inflammation, marked by a reduction in serum transaminase levels. These events are linked with the reduction of a number of immunological suppressive factors (e.g., IL-10, arginase and T-reg frequencies), impacting T cell recovery. Importantly studies have demonstrated a restoration of the balance of Th17/T-regs with reductions of IL-10 and TGF-β upon viral suppression. In a recent study by Nkongolo et al., the intrahepatic compartment was sampled and subjected to scRNA-seq, revealing that a tissue-resident bystander 'hepatotoxic' CD8+ T cell profile was present in patients with active hepatitis which was abrogated on commencing NA therapy, underscoring the important of both viral and immune targets in functional cure for HBV (4). In addition to a robust HBV-specific T cell response, NK cells

have also shown importance in HBV pathogenesis. NA monotherapy does not appear to restore antiviral NK cell function and further adjunct therapy is likely required for this innate boosting. Such findings have also been confirmed in the intrahepatic compartment, where viral suppression resulted in limited changes in NK cell function (5).

Immune modulation with PegIFN-α can offer sustained immune control in a proportion of CHB patients, leading to HBsAg loss and seroconversion at higher rates than that seen with NAs. IFNα is known to activate the innate immune response, Micco et al., demonstrated a potent expansion of activated (HLA-DR+, Ki67+ and TRAIL+) $CD56^{bright}$ NK cells and recovery of their antiviral potential, (IFNγ production) (6). Therapy with PegIFN-α alone, however, does not result in a rapid decline of viral load, thus highlighting its predominant immune modulatory action. Along these lines IFN-α therapy could have dichotomous effects on HBV-specific adaptive immunity. Despite the fact that IFN-α can increase T cell survival, boosting viral antigen presentation and triggering IL-12 production, which might directly rescue the function of exhausted T cells, HBV-specific T cell responses in treated patients may be inhibited by PegIFN-α therapy (6). Notably, a recovery of HBV-specific T cell function is only observed after PegIFN-α therapy cessation in treatment responders (6). As NK cells can negatively regulate HBV-specific T cells, the mechanisms of HBV-specific T cell inhibition during IFNα therapy could be mediated by NK cells (7). This is, however, controversial since in animal models type-I IFN protects T cells from NK cell mediated attack (8). The discordant findings in relation to IFN therapy in CHB dictates that more detailed on-treatment studies are required.

In this current issue of Hepatology, Jiang, Jia, Qian et al., (9) explored the roles of TDF & PegIFN-α therapy in the peripheral compartment of CHB patients using scRNA-seq. They show a differential profile of immune cells in CHB vs healthy subjects, revealing a population of pro-inflammatory monocytes and NK cells in CHB. TDF monotherapy had minimal effects on immune cells compared to TDF+PegIFN-α therapy, suggesting that the regulation of immune cells is primarily due to PegIFN-α. The study identified three CHB-specific cell subsets, pro-inflammatory (Pro-infla) CD14+ Monocytes, Pro-infla CD16+ Monocytes, and IFNG+CX3CR1- NK cells, which correlated positively with HBsAg levels. These subsets could play a crucial role in HBV clearance and hold potential as therapeutic targets. Importantly, pro-inflammatory cytokines, including TNF, IL-1B, IL-6, and IFNG, were found to be increased in CHB patients. TNF was mainly produced by Pro-infla CD14+ Mono and Pro-infla CD16+ Mono cells, with high activation of TNF target genes across multiple immune cells in HBV patients. The TNF signalling pathway could therefore influence immune cell activation, differentiation, and T cell dysfunction during HBV infection. Moreover, PegIFN-α treatment switched the transcriptional profiles of entire immune cells from a pro-inflammatory TNF-driven to an antiviral IFN-α driven pattern.

The study further identified enhanced expression of CXCR4 in all immune cell types, especially in IFNG+CX3CR1- NK cells, suggesting an important role of the CXCL12-CXCR4 pathway in recruiting immune cells to the liver for HBV clearance and the potential utility of CXCR4 as a marker for evaluating inflammation levels. PegIFN-α was able to revert the overall transcriptome of treated samples close to that of the healthy population, diminishing the pro-inflammatory monocytes and increasing IFN-stimulated CD14+ monocytes with a reduction in 'inflammatory score' and the expression of pro-inflammatory genes. This shift in monocyte populations was accompanied by increased expression of antigen presentation-related genes critical for HBV clearance. GSEA analysis revealed that the antigen processing and presentation pathways were enriched in IFN-stimulated CD14+ monocytes, and the proportion of cDC and pDC decreased following PegIFN-α therapy.

As previously reported with *in vivo* studies of PegIFN-α treatment, the proportion of the cytokine producing $CD56^{bright}$ NK cell population increased with PegIFN-α therapy (6), but with no significant increase in the IFNG+CXCR31- NK cells. Naïve B cells increased whilst memory B cells decreased during therapy. Notably, PegIFN-α did not induce T cell differentiation or exhaustion in PBMC of CHB patients and led to an increased proportion of naive and central memory T cells. Following PegIFN-α treatment, DEGs related to naïve and memory T cells, including CCR7, TCF7, IL7R and SELL were increased. PegIFN-α treatment also increased the proportion of CX3CR1+ cells among effector T cells, which have been shown to negatively correlate with the virus burden. Additionally, the study revealed low expression of IFNG and high expression of PRF1 in CX3CR1+ NK and T cells, suggesting their involvement in clearing HBV-infected cells through strong cytolytic abilities.

The application of scRNA-seq in CHB patients undergoing PegIFN-α therapy in this study has unveiled crucial immune subsets and cytokine profiles as potential therapeutic targets. While this study sheds light on the immune landscape of CHB patients and the therapeutic effects of PegIFN-α, there are limitations, including small sample size and incomplete control sample matching along with determining the importance of the immune response during the course of PegIFN-α therapy which has not been addressed. Future studies should involve larger cohorts representing different disease stages, with the use of liver sampling, using fine needle aspirates (10), to further validate the findings and improve our understanding of HBV treatments. More research in this field will undoubtedly contribute to the development of personalized medicine strategies for combating HBV, bringing us closer to the ultimate goal of achieving HBsAg seroconversion and improving patient outcomes.

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