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Sequential Therapy with Tenofovir Plus Peg Interferon Enhances Innate and Adaptive Immunity Compared to Tenofovir Monotherapy in Chronic Hepatitis B Patients

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Abstract

Viral load reduction followed by immunomodulation is an emerging approach to improve the treatment outcomes in patients with Chronic Hepatitis B (CHB). Persistent functional defects in Dendritic Cells (DC) have been observed in CHB patients, even with effective antiviral therapy. We investigated the effects of Tenofovir plus Peg-IFN Sequential Therapy (SQT) on functional restoration of innate and adaptive immunity in CHB patients. HBeAg+ve CHB patients were randomized to receive 48weeks of either tenofovir monotherapy (TM; Gr.1, n=30) or tenofovir with addition of PEG interferon from week 12 to 36 followed by tenofovir sequential therapy (SQT; Gr. 2, n=28) for 48 weeks. Biochemical parameters improved significantly with treatment at week 24 in both groups, but HBeAg seroconversion at week 48 occurred more frequently after SQT (21%) than TM (13%). At week 24, the expression and function of TLR7 and TLR9 in DCs were significantly increased in SQT compared to TM ($p<0.05$). Phagocytic activity of DCs, production of IFN-α and TNF-α by mDCs and pDCs and the expression of specific miRNAs for DC proliferation and maturation like miR155 and miR221, were higher in the SQT ($p<0.05$). After 24 weeks, SQT restored significantly more circulating CD8Tcells ($p=0.02$), CD8+CD127+ Tcells ($p=0.03$) and reduced the PD-1 expression on CD8 T-cells ($p=0.04$) vs. TM. Our results show that in a short period of 24 weeks, SQT significantly improves functionality of DCs. Upregulation of TLR7 and TLR9 and miR155 in DCs by PEG-IFN-α is a novel mechanism that may be quite significant in mounting an effective antiviral response. Influence of longer duration of SQT and immunomodulation needs to be studied.

Keywords: Chronic hepatitis B; Sequential therapy; Tenofovir; Dendritic cells; Toll like receptors

Abbreviations

DC: Dendritic Cells; CHB: Chronic Hepatitis B; ST: Sequential Therapy; TDF: Tenofovir; TLR: Toll like Receptors.

Introduction

Hepatitis B Virus (HBV) infection is one of the most prevalent infectious diseases affecting more than 350 million subjects worldwide. About 5% of acutely infected HBV patients develop chronic infection due to ineffective host immune response [1,2].

Immunological responses to chronic hepatitis B (CHB) are characterized by an expansion of CD4 and CD8 T cells with minimal HBV specific response [3]. Enhancement of dendritic cell (DC) function is believed to play a vital role in priming CD4 and CD8 T cells to mount an effective antiviral response against HBV [4]. DCs recognise HBV antigens or viral DNA through Toll Like Receptors (TLRs) and activate NF-κB, p38 MAPK signalling pathway resulting in secretion of type-I Interferon and pro-inflammatory cytokines as part of antiviral adaptive immune response [5]. Functional defects in DCs result in CD4 and CD8 T cell hypo-responsiveness in CHB patients [6,7]. The DC dysfunction is probably a consequence of HBV viremia as reduction in HBV viral load results in an improved immunologic phenotype [7].

Antiviral therapy with tenofovir results in partial reconstitution of effective T cell responses due to a reduction in exhaustive cells [8]. Interferon based immuno-modulatory therapy induces innate immunity and yield strong protective immunity in CHB patients [9,10]. IFN plays an immunoregulatory role in induction, activation and maturation of macrophages and DCs, resulting in increased cytotoxic T-cell activity for lysis of infected hepatocytes. IFN monotherapy in HBeAg-+ve CHB patients can achieve HBeAg seroconversion in about 20-40% of patients and loss of HBsAg in approximately 8% of patients [11]. While a lot of work has been done on the mechanism of immunomodulation by intreferon therapy in CHB, its role in modulating DCs to activate effector CD8+ T cells needs more attention [12].

Since oral antiviral agents and immunomodulators have different mechanisms of action, though overlapping to some extent, it is logical to evaluate a combination of them for better treatment outcomes [13]. However, the results using combination of interferon with antiviral agents have shown mixed results [13-15]. There have been very few studies which have utilized the potential of immune restortaion after viral load reduction, with modulation by interferon therapy [14,15].

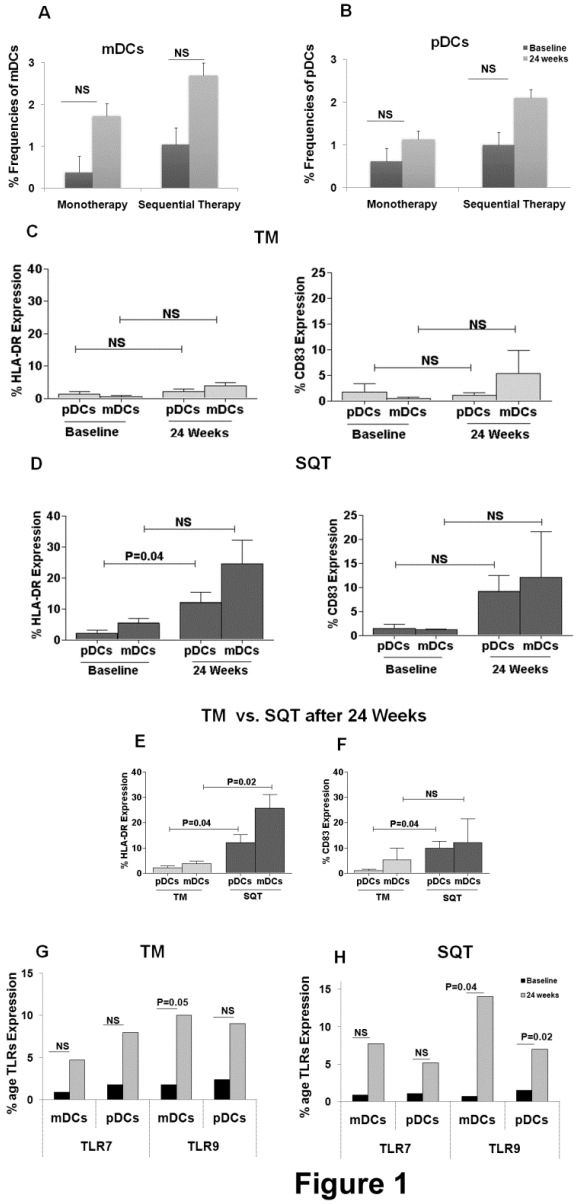

Figure 1: Improved phenotype and functionality of mDCs and pDCs in Peripheral blood among patients receiving monotherapy and sequential therapy. (A-B) No significant increase in frequencies of mDCs and pDCs was observed after 24weeks of both therapies. (C-D) Expression of co-stimulatory molecules was analyzed after 24week of each therapy. No significant difference was observed with both therapies. (E-F) In between two therapies, sequential therapy increased the HLA-DR expression on mDCs and pDCs and CD83 expression on pDCs. (G-H) With TLRs agonist stimulations, in sequential therapy mDCs and pDCs showed significantly increased expression of TLRs. Results were expressed as the mean and error bars represent SD.

Both the studies have shown that sequential therapy has improved HBeAg serconversion rate as compared to monotherapy. However, in these studies, lamivudine was used as an antiviral agent. A comparison with potent agents such as tenofovir has not been investigated.

The precise immunological mechanisms underlying the enhanced response with sequential therapy have not been studied. It is hypothesized that restoring the function of DCs may be the major mechanism for immune restoration. Therefore, we undertook to compare the immunological responses during tenofovir monotherapy and sequential therapy with tenofovir followed by peg interferon therapy in CHB patients.

Materials and Methods

Patients

Treatment naive CHB patients, HBeAg positive and with raised ALT (> 48 IU/ml) for at least 6 months were screened for inclusion in the trial at the outpatient clinic of the Institute of Liver and Biliary Sciences (ILBS). Patients with ALT levels of >10 times ULN and those with clinical or radiological evidence of cirrhosis of the liver, co-infection with HCV, HDV or HIV, haemoglobin less than 10 g/dL, low platelet (<100,000/mm^3) or leukocyte counts (<3,000/mm^3), contraindications to interferon therapy, pregnant or nursing mothers were excluded from the study. Subjects with a prothrombin time prolonged by more than 3 seconds, a serum albumin level <2.5 g/dL, or evidence of liver disease because of other etiology; serum creatinine >1.2 times ULN, history of organ transplantation; serious concurrent medical illnesses (like malignancy, severe cardiopulmonary disease, uncontrolled diabetes mellitus, alcoholism) and inability to give informed written consent were excluded from the study. The clinical trial was approved by the institutional Ethics committee and all patients signed informed consent.

Patients with HBV DNA levels >2×10^4 IU/ml underwent a liver biopsy to confirm the diagnosis of chronic hepatitis B. Patients fulfilling the selection criteria were randomized in an open label manner into either of the two groups:

Group 1 (n=30): Tenofovir Monotherapy (TM): Patients received tenofovir alone 300 mg daily for 48 weeks.

Group 2 (n= 28): Tenofovir plus Peg-IFN in a sequential manner, the Sequential therapy (SQT):

In the sequential therapy, the patients received Tenofovir alone for 12 weeks and from week 13th, they received Peg-IFN-α 2b, 1.5 mcg/kg once a week subcutaneously, along with tenofovir till week 36 and then from week 37th till week 48, they received Tenofovir alone (Supplementary Figure 1).

Laboratory evaluation

HBV DNA levels, HBsAg, HBeAg and antibody to HBeAg (anti-HBe) were determined at baseline and at week 4, 12, 24, 36 and 48 after initiating treatment.

End-points

The primary end-point of the study was absence of detectable HBV DNA and secondary end-points were loss of HBeAg, appearance of anti-HBe (HBeAg seroconversion) and normalization of ALT (≤40 IU/L) at week 48.

Quantitation of HBV DNA

HBV DNA was extracted from 100 µl of plasma and quantification of HBV DNA was done using COBAS® AMPLICOR HBV MONITOR kit (Roche diagnostic, USA) as described previously [7].

Analysis of mDCs and pDCs by flow cytometry

The study of dendritic cells at different stages of antiviral therapy in the two groups was one of the main objectives. The mDCs and pDCs were isolated and characterized in the peripheral blood by using anti-CD11c-PeCY7, anti-DCSIGN-PE (BD Pharmingen, CA, USA) and anti-BDCA2-FITC, anti- CD123-PE, anti-CD83-PE, anti-HLA-DR-APC (eBiosciences, CA, USA). CD3 and CD8 T cells and expression of PD1 on T cells was analyzed using anti-CD3- PeCy7, -anti-CD8-APC, anti-PD1- FITC antibody (BD Pharmingen,CA, USA) for 20 minute at room temperature in the dark, and then washed twice with PBS and fixed using 0.1% paraformaldehyde. 30,000 events for T cells and 80,000 events for DCs were acquired on DAKO flowcytometer. Data was analyzed using Flow Jo software version 8.7.1 from Treestar, USA.

In-vitro generation of DCs from PBMCs and their phagocytotic activity

PBMCs were suspended in RPMI1640 medium supplemented 5% fetal bovine serum (FBS) in 6 well plastic plates for 3hrs. The non-adherent cells were gently removed and the adherent cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 10 ng/mL rhGM-CSF, and 5 ng/mL rhIL-4 (Sigma, USA) in a humidified atmosphere at 37°C in CO_2 incubator.

After three days, immature DCs were seeded in a 6 well plate at 5×10^5 cells/ well and incubated with 1mg/ml BSA FITC (BD Pharmingen, USA) for 30 minutes. At the end of incubation, cells were washed with 1X PBS twice. The degree of phagocytosis was analyzed by FACS Caliber flow Cytometer. Data was analyzed using Flow Jo software.

Stimulation of DCs by Different TLR agonists

To determine the frequency of TNF- α and IFN- α production by DCs, mature DCs were plated, in the presence of different TLR agonists (TLR7/8, TLR9; 1 µg/ml each (Invitrogen, CA, USA). After the first 1 h of incubation, Brefeldin-A (Sigma, St Louis, MO, USA) at a final concentration of 2 µg/ml was added and cells were incubated further for five hours in CO_2 incubator. After incubation, cells were washed with 1x PBS, centrifuged, permeabilized, fixed, and stained with APC anti-CD11c, FITC anti- BDCA2 , PE anti- TNF-α or PE anti- IFN-α (BD Pharmingen, CA, USA). After staining, the cells were analyzed for flow-cytometric analyses. Data was analyzed using Flow Jo software version 8.7.1 from Treestar, USA.

Isolation of PanDCs and Total RNA extraction

Approximately 20-25 ml of blood sample was processed from the patients at baseline and at 24 weeks for the isolation of DCs using magnet assisted cell sorting Pan DC enrichment kit (Stem Cell Technologies, USA). Total RNA was isolated from isolated Pan DC+ve fraction using miRvana kit (Ambion, Life technologies, Bangalore, India).

Quantitative expression of miRs in dendritic cells

The cDNAs were prepared using universal cDNA synthesis kit (Exicon, San Francisco, USA). LNA™ PCR amplification was performed using MicroRNA LNA™ PCR primer sets of miRs- 146, 155, 221 and 222 and ExiLENT SYBR® Green master mix. SYBR® Green is used for detection of amplification. Quantitative expression of miR was performed in triplicate in ABI Via7 RT- PCR machine (Invitrogen, USA). Amplification of 5s-rRNA was used as the control for normalization.

Statistical analysis

We have compared various immunological parameters at baseline and at 24 weeks of therapy in both groups as well as between two groups of monotherapy and sequential therapy. Data were analyzed with SPSS17.0 statistical software. The comparison between groups was analyzed using Mann Whitney Test or t-test as appropriate for continuous data. Qualitative data was analyzed using chi-square test wherever applicable. Besides this, Wilcoxon signed rank test was used to see the decreasing viral load in both the therapies. A p value < 0.05 was considered statistically significant.

Results

The clinical characteristics of the 58 patients with chronic hepatitis B enrolled in the study are shown in Table 1. There were no significant differences in the age, serum bilirubin, albumin, ALT, and serum HBV DNA levels between the two. All the patients were HBeAg positive.

Parameter	Tenofovir Monotherapy (TM, Gr. 1, n=30)	Peg-IFN plus Tenofovir Sequential Therapy (SQT, Gr. 2, n=28)	P Values
Age (years) mean ± SD	35.4± 15	29.9 ± 11.9	P=0.13 (NS)
Sex (M:F)	22:08	24:04:00	P=0.24 (NS)
Median Serum bilirubin (mg/dl; range)	0.7 (0.5-1.2)	0.75 (0.4-1.5)	NS
Median Albumin (g/dl ; range)	4.3 (0.8-4.7)	4.35 (3.7-5)	NS
Median ALT (IU/L; range)	60.5 (45-230)	62.5 (45-205)	NS
HBV DNA (mean ± SD; log10 copies/ml)	6.26 ± 1.25	6.61± 1.16	P=0.33 (NS)

Table 1: Baseline Clinical and Virological Characteristics of patients

Improved HBeAg Seroconversion with SQT

After initiation of the therapy, there was >2 log reduction in HBV DNA levels in both groups by 12 weeks and normalization of ALT (≤40 IU/L) at week 48.There was loss of HBeAg and appearance of anti-HBe (HBeAg seroconversion) with both therapies. However, in the SQT, HBeAg seroconversion occurred more frequently than in TM (21% than 13%) at week 48 (Table 2).

Groups	HBV DNA (log IU/ml)				eAg Seroconversion (%)			
	Baseline	12 Wk	24 Wk	48 Wk	Baseline	12 Wk	24 Wk	48 Wk
Mono-Therapy TM (n= 30)	6.26 ± 1.25	2.7± 1.3	1.82 ± 1.2	1.65 ± 0.69	-	13%	13%	13%
Sequential Therapy SQT (n=28)	6.61± 1.16	3.59 ± 1.4	2.1 3± 1.5	1.48 ± 0.9	-	13%	21%	21.40%

Table 2: eAg Seroconversion During Mono-Therapy and Sequential Therapy.

Improved innate immune functionality with sequential therapy

Frequencies of myeloid DCs and plasmacytoid DCs: Frequencies of mDCs and pDCs in the peripheral blood were examined in CHB patients before and after 24 weeks of therapy. Compared to the baseline, there was a modest increase in the frequencies of mDCs and PDCs after 24 weeks of therapy in both the groups (TM; Baseline to 24 wk therapy; mDCs 0.4 ± 0.2% to 1.72 ± 0.9%, pDCs 0.6 ± 0.2% to 1.1± 0.4%, P=NS) (SQT; mDCs 0.6 ± 0.3% to 2.7 ± 1%, pDCs 1 ± 0.4% to 2.14 ± 0.7%, p=NS, Figures 1A and 1B).

Increased Co- stimulatory markers in DCs with Sequential Therapy: HLA-DR and CD83 are markers of mature dendritic cells. To define whether drug therapies in CHB patients are associated with functional alterations of DCs, expression of HLA-DR and CD83 was analyzed before and after 24 weeks of therapy.

With SQT, there was a significant increase of HLA-DR expression in pDCs from baseline to 24 weeks (1.0 ± 0.3 vs. 10 ± 2, P=0.04) but not in mDCs (3 ± 1 vs. 22 ± 2, P=NS). There was a moderate but not a significant increase in the expression of CD83 in pDCs and mDCs with SQT (pDCs; 1 ± 0.5 vs. 8 ± 2.1, P=NS; and mDCs; 0.9 ± 0.1 vs. 12 ± 3.04, P =NS, Figures 1C and 1D).

With the tenofovir monotherapy however, there were no changes in the expression of both HLA-DR and CD83 on DCs from baseline to 24 weeks.

When results of both regimens were compared (SQT vs. TM) at 24 weeks, HLA-DR expressions were significantly increased with SQT than TM in pDCs (11.0 ± 2 vs. 1.5 ± 0.5 P =0.05) and mDCs (22 ± 2 vs. 3 ± 1, P=0.02, Figure 1E). Similarly, CD83 expression was also increased in pDCs with SQT than with TM (10 ± 2 vs. 1 ± 0.5 v, P=0.04, Figure 1F).

Increased Toll like receptor expression in DCs with Sequential Therapy: TLRs recognize pathogen derived products and further provoke DC maturation and antigen presentation to naïve T cells. We examined the expression of TLR7 and TLR9, which specifically express in pDCs and mDCs. With both therapies, there was marginal increase in expression of TLR7 and TLR9 in DCs at the end of 24 weeks, importantly, with SQT, change in TLR9 expression in mDCs and pDCs, was more significant than TM (P=0.04 and 0.02 respectively, Figures 1G and 1H).

Effect of Sequential Therapy on phagocytic activity of DCs: In order to address, whether the increased expression of co-stimulatory molecules such as HLA-DR, CD83 and increased expression of TLR7 and 9 on DCs was related to improved functionality of DCs, we assessed *in vitro* generated DCs for their phagocytic activity using a novel BSA uptake assay. In this assay, BSA was labeled with FITC and DCs functional ability was determined by the uptake of BSA molecules by DCs.

The ability of DCs to uptake BSA FITC molecule significantly increased from baseline values of 9.5% to 61% with SQT, but only to 22% with TM at week 24 (P=0.005, Figure 2A). We measured the median fluorescence intensity (MFI) of BSA FITC molecule used by DCs. With ST, after 24 week, DCs showed 300-fold increase in MFI of BSA FITC molecule from baseline compared to only 50 fold increase with TM (P=0.02, Figure 2B).

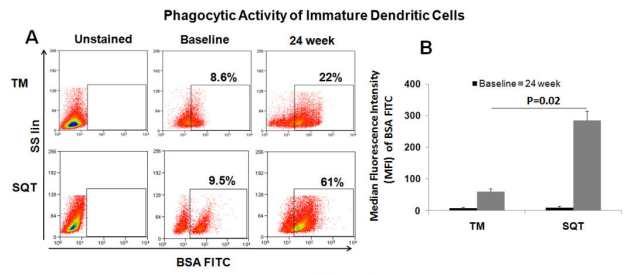

Figure 2

Figure 2: Phagocytic Activity of Immature Dendritic Cells. (A) Representative dot plot showing BSA FITC uptake by dendritic cells at baseline and after 24 week of monotherapy or sequential therapy. Increased BSA FITC uptake was observed by dendritic cells in sequential therapy. **(B)** Increased median fluorescence intensity of BSA FITC molecule after 24 week of sequential therapy.

TLR stimulation increases the Pro-inflammatory Cytokines (TNF-α and IFN-γ) production by DCs: Next, we evaluated whether DCs respond to *in vitro* TLR7/8, TLR9 agonist stimulations, which lead to increased secretion of TNF-α and IFN-γ pro-inflammatory cytokines. DCs isolated from whole blood from subjects in both groups at baseline and at 24 weeks were stimulated with TLR7 and TLR9 agonists and we measured the levels of IFN-γ and TNF- α cytokine

production. At 24 weeks of SQT, stimulation with TLR7 and TLR9 agonists significantly induced the IFN-γ and TNF-α production by pDCs and mDCs (P<0.05, Figure 3). However, TM did not result in an increase of TNF-α and IFN-γ at the end of 24 weeks (Figure 3).

Increased expression of miR- 155 and miR-221 in dendritic cells with sequential therapy: We further investigated the mechanisms that could be responsible for mediating the improved phagocytic and cytokine production by DCs at week 24 week of therapy. . In this regard, we analyzed the levels of miRNAs that could be directly responsible for improved DC functionality.

We analysed specific candidate miRNAs namely 146a, 146b, 155 and 221, which are involved in mediating DC proliferation and maturation. Out of four miRNAs, miRNA 155 and 221 were found to be influenced during therapy. After 24 weeks of SQT, expression of miR 155 and miR 221 were significantly increased from baseline (P<0.05, Figure 3C) compared with patients receiving TM.

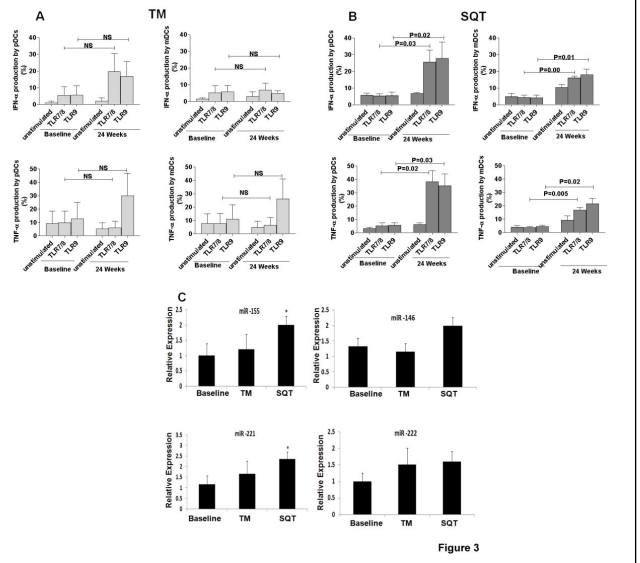

Figure 3

Figure 3: TLR agonist stimulations increased the secretion of Pro-inflammatory Cytokines (TNF-α and IFN-γ) by DCs. (A-B) TLR7/8 and TLR9 agonist was used to stimulate DCs at baseline and after 24 week of mono-therapy and sequential therapy. Mono-therapy showed no significant increase in the production of IFN-α and TNF-α secretion; however, there was significant increase in IFN-α and TNF-α secretion with sequential therapy. (C) Relative expression of miR 155, 146, 221 and 222 in DCs at baseline and after 24 week of mono-therapy and sequential therapy; miR-155 and miR-221 expression in DCs was increased in sequential therapy.

Restoration of adaptive immune response with sequential therapy

With 24 weeks of either TM or SQT therapy, frequencies of CD8+ T cells and CD8+CD127+ memory T cells were increased compared to baseline (Table 3, Figure 4A and 4B). However, using SQT, there was a significant decrease in the PD-1 expression on CD8+T cells from base line (45% vs. 4%, P=0.009, Figure 4C and 4D). This was not seen in patients receving TM (63% vs. 42%, P=NS).

Therapy	% Increase in frequency of CD8+ T cells (week 24 vs. Baseline)	% Increase in frequency of CD8+ CD127 +T cells (week 24 vs. Baseline)	% Decrease in frequency of CD8+PD1+ T cells (week 24 vs. Baseline)
TM	10 ± 2	36 ± 7	17± 4
SQT	16 ± 3.2	45 ± 9	45.5 ± 8.4
P values	NS	NS	P<0.05
% increase or decrease was expressed as mean ± SEM			

Table 3: Increased CD8+ and CD8+CD127+ T cells and decreased CD8+PD1 with Sequential Therapy

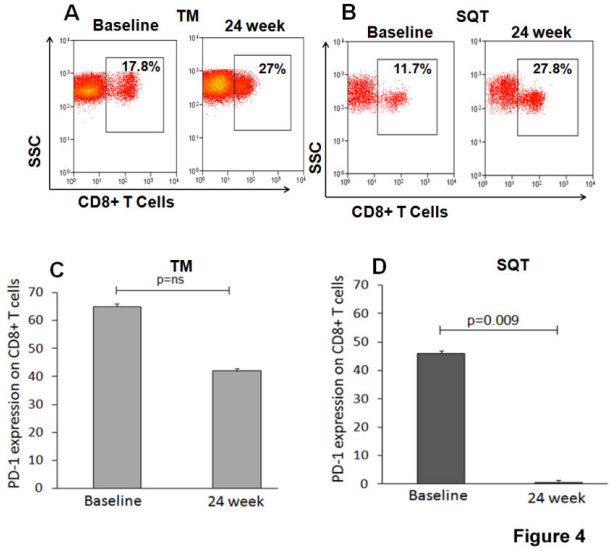

Figure 4

Figure 4: Adaptive immune response during both therapies. (A-B) Representative dot plots showing no significant increase in the percentages of CD8+ T cells after 24 week of mono-therapy or sequential therapy. **(C-D)** PD-1 expression was analyzed during both therapies; with sequential therapy PD1 expression on CD8+T cells was decreased.

Discussion

Results of the present study, undertaken to investigate the immune mechanisms underlying immunomodulation with antiviral therapies for chronic hepatitis B show that sequential therapy resulted in functionally more robust DC phenotypes which probably results in enhanced restoration of CD4 and CD8 T cells by week 24 as compared to tenofovir monotherapy. Our data also demonstrates that with SQT, miR 155 and miR 122 expressions was increased in DCs, these micro RNAs only express during proliferation and activation of DCs. Therefore, increased expression of miR 155 and miR 122 are the basis for improved dendritic cell functionality in SQT.

We have earlier demonstrated tenofovir therapy achieves a 2 log HBV DNA reduction by week 12, but HBV specific adaptive T cell responses improved significantly only by week 24 [8]. Therefore, in the

present study, we introduced a strong immuno-modulator (PEG-IFN-α) after 12 weeks of tenofovir monotherapy and compared the effects of combination sequential therapy using PEG-IFN to tenofovir monotherapy at week 24; i.e., 12 weeks after giving a combination. We correlated this with the clinical and virological outcomes.

Several studies have demonstrated that HBsAg abrogates TLR7 and TLR9 expression on monocyte-derived DCs, decrease DC functionality, reduce expression of HLA-DR, CD86, CCRs and ICAM-1, impair antigen presentation ability, and diminish the capacity to secrete IL-12 and IFN-γ [4-7,16]. Targeting improved DC function has been of immense interest in the area of HBV therapeutics.

There is evidence showing that both mDC and pDC are functionally impaired in patients with chronic hepatitis B [17,18]. Antiviral treatment with adefovir improves the number and functionality of mDCs, but not of pDCs and hence provides clues on why current antiviral therapy does not lead to consistently sustained viral eradication. Anti-viral therapy with telbivudine in CHBV patients also have shown markedly enhanced expression of HLA-DR on monocyte-derived DCs with increased capacity to produce interleukin (IL)-12 [19]. Treatment with ribavirin also induced the activation of pDCs and mDCs in chronic HBV patients [20]. Further, *in vitro* cell based assays showed, DC activity was enhanced by use of CpG-ODNs [21], HBsAg-activated dendritic cells [22] or by immunization with dendritic cells pulsed HBV particles [23,24] and TLR7 agonist [25]. However, a direct relationship between improved immunity with antiviral treatment and clinical outcomes such as eAg seroconversion has not yet been established until now. Our results show that with sequential therapy there were improvements in the frequency and phenotype of DCs; as measured by increased co-stimulatory molecules like HLA-DR and TLR 7 and 9 expressions on DCs. Importantly, there was enhanced phagocytic activity of DCs with SQT, which was proven by uptake of BSA by DCs. On improvement of DCs functionality, IFN-γ and TNF-α cytokine secretion was also improved in patients receving the SQT.

Dendritic cell activation was under the tight regulation of several molecules including many miRNAs [26-28]. Recent studies have demonstrated several specific miRNAs contribute to DC differentiation and proliferation [29-31]. Differentiation of immature DCs to mature DCs is regulated by 27 stage-specific miRNAs and capable of priming an effective antiviral immunity [31]. Recent evidence also suggested that these miRNAs may directly regulate IFN-β protein expression and contribute significantly to the regulation of IFN in innate immune responses [32]. Therefore, in our study we have investigated four DC proliferating miRNAs and found that the improved functionality of DCs was associated with increased expression of miRNAs specific for proliferation and expansion of DCs. Our results showed that with sequential therapy improvement in functionality of DCs was associated with increased expression of miR 155 and miR 221 specific for DC proliferation.

Defects in innate immunity influence further adaptive immunity and we have analysed few of the adaptive immunity parameters after both therapies. Before any treatment, adaptive immune response is generally depressed in CHBV patients due to increase in T regulatory cells, increase of PD1 expression in CD8 T cells [33]. PD1 expression is exhaustion marker and increased presence of PD1 indicates exhaustive CD8+T cells. In our previous study *in vitro* PD-1 blockade strategy in CD8+ T cells and Tregs have shown improved survival of HBV-specific CD8+ T cells [8,34]. In present study also with sequential therapy, there was steep decrease in PD1 expression on CD8+T cells and increase in effector T cells. Therefore, in our study, temporal association of these changes in DC function to a decline in exhausted PD-1 expressing CD8+T effector cells suggesting improvement in innate-adaptive cross talk among those individuals receiving sequential therapy (Figure 5).

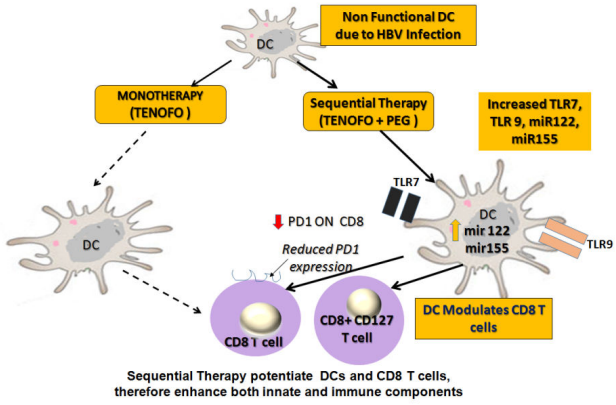

Figure 5: Improved Innate and adaptive immunity with sequential Therapy. Sequential Therapy potentiates DCs which further modulate CD8 T cells. SQT also helps in reducing the PD1 expression on CD8T cells which make them functional, therefore SQT enhances both innate and adaptive immune components.

Sequential therapy with Tenofovir and PEG IFN provided the unique dataset showing prolific anti-HBV immunity with effective HBV suppression and immune modulation. Since similar results were not observed with tenofovir monotherapy, a distinct role for immunosuppressive therapy coinciding with HBV suppression is vital to achieve optimal therapeutic response. In conclusion, treatment regimens that combine potent antiviral agents and immune modulators, such as IFN-alfa or TLR7 agonist need to be investigated to reduce the need for life-long directly acting antiviral therapy in CHB patients.

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Authors Contribution

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Provision of study patients: Shiv Kumar Sarin

Critical revision of the article for important intellectual content: NirupmaTrehanpati, Shiv Kumar Sarin and Shyam Kottilil

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Conflicts of Interest

There is no conflict of interests for all authors

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