Uptake and T Cell Stimulation by BJT-778-HBsAg Immune Complexes: Insights into Anti-HBs Monoclonal Antibody Function

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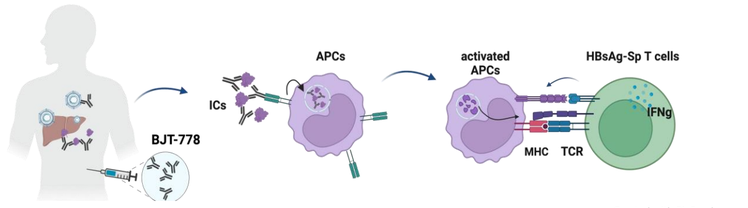
Introduction

- In chronic hepatitis B patients, the sustained presence of a high quantity of hepatitis B virus surface antigens (HBsAg) contributes to the exhaustion of HBV-specific T and B cell responses.
- BJT-778 is a fully human IgG1 monoclonal antibody targeting the antigenic loop of HBsAg and is currently in clinical development for Chronic Hepatitis B and D.
- BJT-778 is designed to bind HBsAg and reduce its antigen load, while simultaneously enhancing antigen presentation and immune activation to improve disease outcomes.

Hypothesis: The addition of the BJT-778 anti-HBs mAb to HBsAg results in the formation of ICs, facilitating enhanced Fc receptor-mediated uptake of HBsAg by monocytes and dendritic cells. This process improves antigen presentation, leading to amplified activation of HBV-specific T cells.



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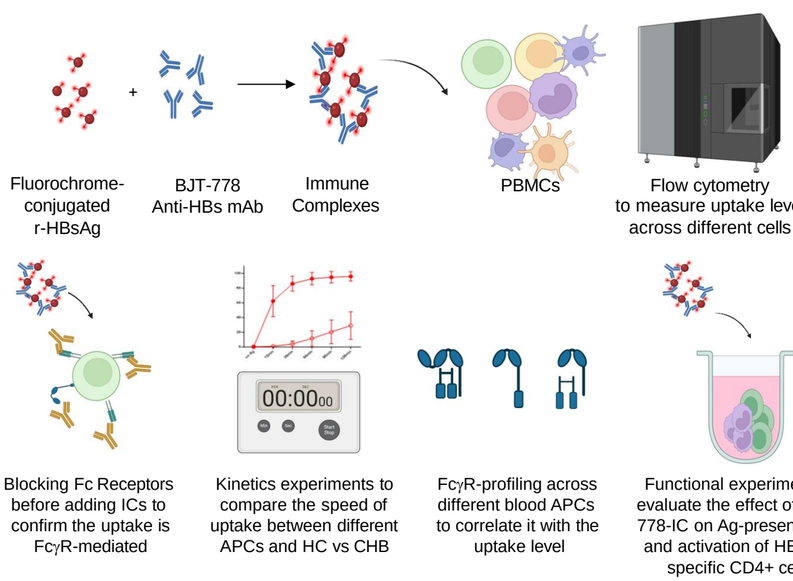
Aims:

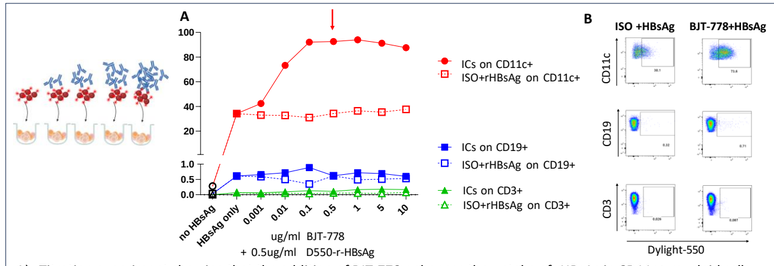
- To evaluate the ability of BJT-778 to enhance the uptake of HBsAg immune complexes.
- To identify the primary cell types responsible for internalizing HBsAg in PBMCs.
- To investigate the role of Fc receptors in mediating immune complex uptake by APCs.
- To assess the difference in IC uptake between APCs from CHB patients and healthy controls.
- To assess the impact of BJT-778-mediated HBsAg uptake on HBsAg-Specific T cell activation.

Methods



Results

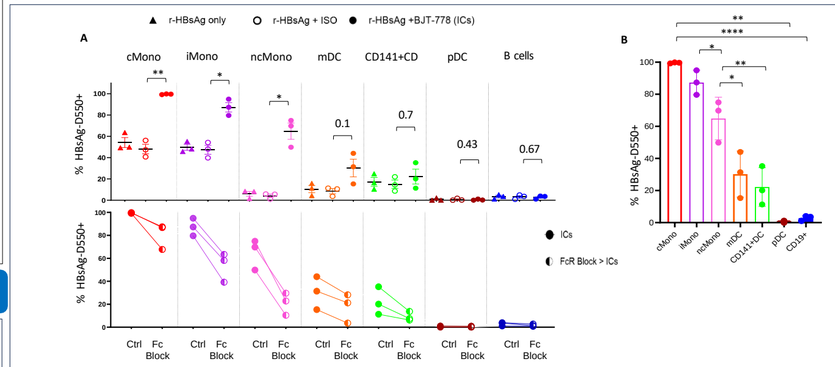
Figure 1. BJT-778-ICs enhance the uptake of HBsAg by blood myeloid cells.



A) Titration experiment showing that the addition of BJT-778 enhances the uptake of rHBsAg in CD11c+ myeloid cells. ICs = Immune complexes consisting of BJT-778 and rHBsAg; ; ISO = Isotype Ab. Optimal concentration = 0.5 ug/ml rHBsAg : 0.5 ug/ml BJT-778.

B) Representative flow cytometry plots showing HBsAg uptake in CD11c+, CD19+ and CD3+ cells in isotype vs ICs conditions.

Figure 2. A distinct hierarchy of IC uptake exists among different blood APCs, which is blocked by inhibiting FcγRs.

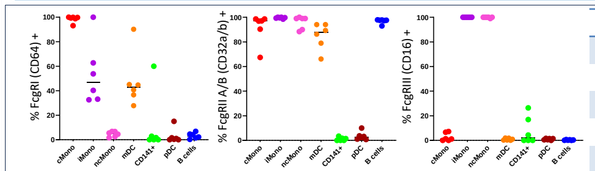


A) % positivity for HBsAg-D550 on different cell subsets was measured by flow cytometry. cMono, iMono, and ncMono refer to Classical, Intermediate, and Non-classical monocytes, respectively. mDC: Myeloid dendritic cells. pDC: Plasmacytoid dendritic cells.

B) Average % uptake of r-HBsAg-D550-ICs across different blood cell subsets from 3 healthy donors. Mann Whitney t-test was used for the statistical analysis.

C) Validation of FcR-mediated uptake of HBsAg-ICs on PBMC samples of HC individuals. Cells were pre-incubated with FcγR receptor blocker before adding HBsAg-ICs.

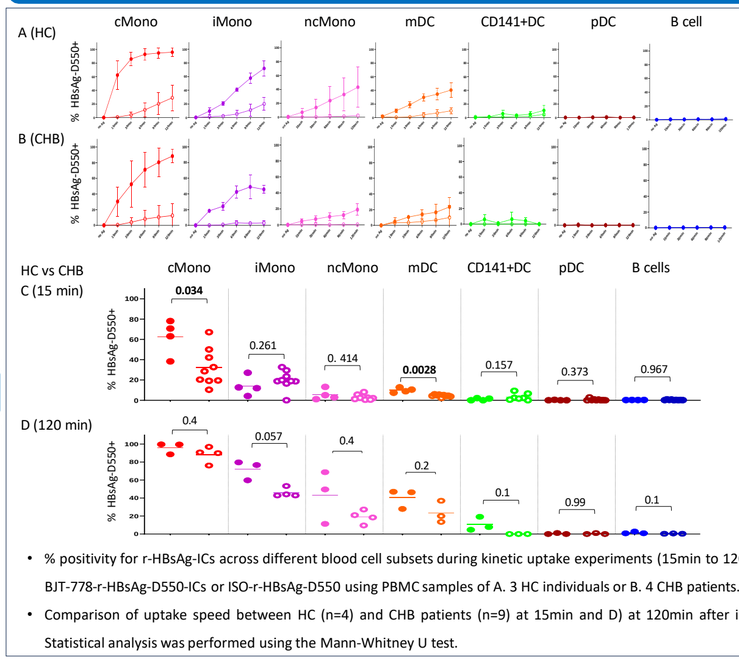
Figure 3. The level of HBsAg-IC uptake correlates with expression profile of activating FcγRs (CD64 and CD16) on various APCs and the binding affinity of BJT-778.



Fcγ Receptors	Binding Affinity (Kd, M)
FcγRI (CD64)	4.57E-09
FcγRIIIA (CD16A)	8.98E-07
FcγRIIIB (CD16B)	5.56E-06
FcγRIIA (CD32A)	6.02E-06
FcγRIIB (CD32B)	1.94E-05

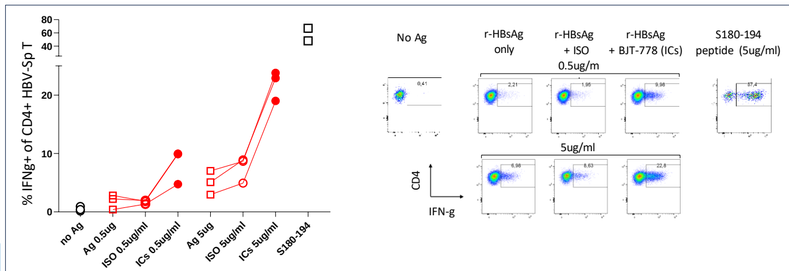
- The expression profile of FcγRI, FcγRII and FcγRIII was assessed on PBMC samples of 5 different CHB patients and one HC individual using flowcytometry to correlate it with HBsAg-IC uptake.
- Cell types showing a significant enhancement in HBsAg uptake with ICs, express high levels of activatory FcγRI (CD64) or FcγRIII (CD16). Available Abs cannot distinguish human CD32a from CD32b.
- Affinity profile of BJT-778 to FcγRs assessed using a Biacore T200 instrument shown in the table.
- Data suggests immune complex uptake is driven by FcγR1 and FcγR3

Figure 4. IC uptake is significantly slower in cMono and mDCs from CHB patients compared to healthy controls.



- % positivity for r-HBsAg-ICs across different blood cell subsets during kinetic uptake experiments (15min to 120min) with BJT-778-r-HBsAg-D550-ICs or ISO-r-HBsAg-D550 using PBMC samples of A. 3 HC individuals or B. 4 CHB patients.
- Comparison of uptake speed between HC (n=4) and CHB patients (n=9) at 15min and D) at 120min after incubation. Statistical analysis was performed using the Mann-Whitney U test.

Figure 5. BJT-778-rHBsAg-ICs uptake by CD14+ monocytes enhances activation of HBsAg-specific T cells.



- % IFN-γ positivity among HBs180-193-specific CD4+ T cells after overnight co-culture with CD14+ Monocytes of an HLA-DR11+ HC individual incubated with either 0.5 or 5 ug/ml of r-HBsAg alone, r-HBsAg-ISO or r-HBsAg-BJT-778-ICs.
- Cells without the addition of HBsAg and S180-194 were utilized as additional negative and positive controls, respectively.

CONCLUSION

- These data demonstrate that BJT-778 forms ICs with HBsAg and facilitates enhanced uptake of HBsAg in professional antigen presenting cells – likely in vivo targets of this response.
- Enhanced uptake of HBsAg resulted in increased HBV-specific CD4 T cell activation.
- Overall, these data support a dual mechanism of action through removal of circulating HBsAg and stimulation of HBV-specific T cell immunity.

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