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Evaluation of an optimized HBV nanocapsule vaccine formulation for neonates

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Introduction

350 million people are chronically infected with the Hepatitis B Virus. One common route of HBV infection is the mother-to-child transmission. 90% of infected neonates develop chronic hepatitis. The lack of efficient treatment underlines the need of novel approaches. Aim of the present study was the large-scale expression and purification of hepatitis B surface antigen (HBsAg) out of the yeast *Pichia pastoris* and the synthesis of HBsAg-nanocapsules. The stimulatory effect of different TLR ligands was evaluated and a human coculture model with neonatal monocyte-derived dendritic cells (moDCs) and autologous T cells was established. The immunogenic properties were evaluate after HBsAg-stimulation and maturation of moDCs with the adjuvant monophosphoryl lipid A (MPLA) and IFNγ.

Summary & Conclusion

- **Phenotypical maturation and proinflammatory cytokine secretion after incubation of neonatal moDCs with MPLA + IFNγ**
- **Intracellular expression of hepatitis B surface antigen (HBsAg) results in self assembled virus like particles (VLPs)**
- **Synthesis of polymeric nanocapsules (NCs) out of HBsAg**
- **Uptake of HBsAg-NCs by human moDCs is enhanced by MPLA-coating onto the NCs**
- **HBsAg-stimulation and MPLA + IFNγ maturation of moDCs, derived from cord blood, increased T cell proliferation and IFNγ secretion**

Results

1 (A) CD80 Expression — Cord Blood moDCs — IL-12 Secretion (B)

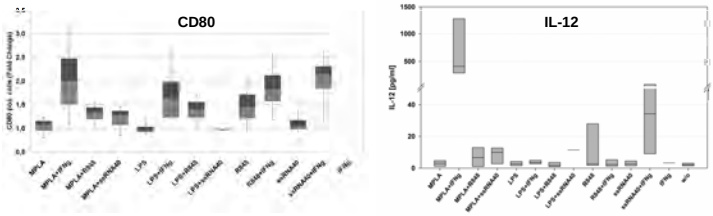
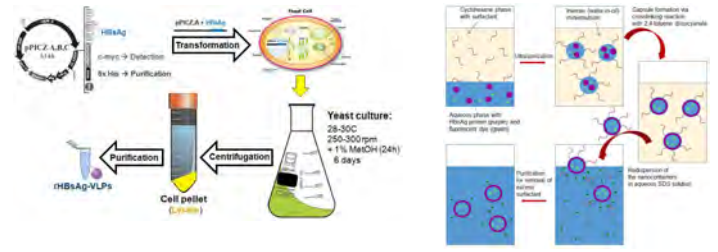


Figure 1: (A) Phenotypic Characterization of neonatal moDCs and (B) IL-12 Secretion after TLR ligands stimulation. Neonatal moDCs were incubated with 1µg/ml MPLA or LPS (TLR 4 Ligand) and R848 or ssRNA40 (TLR 7/8 Ligand) with or without IFNγ (50ng/ml) for 24h. Flow cytometry was performed to verify the expression of costimulatory molecules, e.g. CD80. Cytokine release was determined using ELISA , e.g. IL-12p70.

2 (A) HBsAg Protein Expression — (B) Synthesis of HBsAg Nanocapsules



(C) Characterization of HBsAg–VLPs and HBsAg–Nanocapsules (NCs)

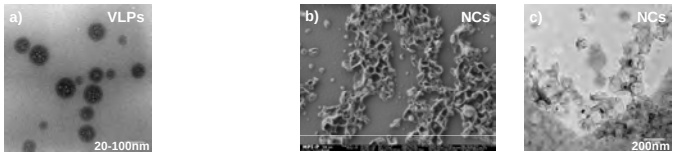


Figure 2: (A) Scheme of HBsAg production and (B) HBsAg-Nanocapsules Synthesis. (A) The HBsAg gene was inserted in pPICZ.A vector (Invitrogen), the constructed expression plasmid was linearized and transformed into KM71H yeast cells. The recombinant strain was cultured at 29°C with constant shaking in baffled flask containing BMMY medium with 1% methanol. Induced cells were harvested, centrifuged and disrupted via glass beads. The recombinant HBsAg was further purified out of the cell lysate by Ni-NTA affinity chromatography and the resulted HBsAg particles were visualized by (C; a) transmission electron microscopy (TEM), (B) HBsAg-nanocapsules were synthesized using the inverse miniemulsion process. NCs were encapsulated with cy5-labeled oligonucleotides and coated with MPLA . (C; b) TEM and (C; c) scanning electron microscopic (SEM) images of the HBsAg-NCs were shown.

3 HBsAg–Nanocapsules Uptake

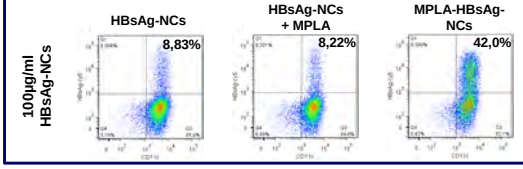


Figure 3: Uptake of HBsAg-Nanocapsules (NCs) by neonatal moDCs. Flow cytometric analysis was performed to quantify the uptake of nanocapsules with soluble MPLA or MPLA-coated NCs after 4h.

4 moDCs + T cells – Coculture Model

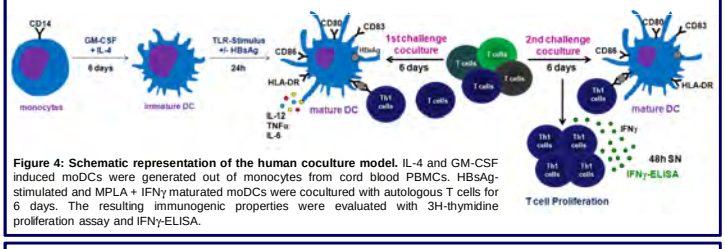
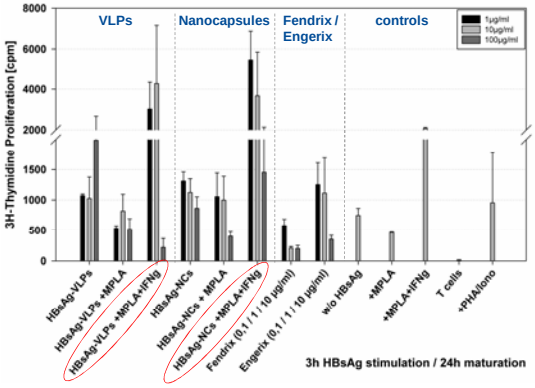


Figure 4: Schematic representation of the human coculture model. IL-4 and GM-CSF induced moDCs were generated out of monocytes from cord blood PBMCs. HBsAg-stimulated and MPLA + IFNγ maturated moDCs were cocultured with autologous T cells for 6 days. The resulting immunogenic properties were evaluated with 3H-thymidine proliferation assay and IFNγ-ELISA.

5 (A) T cell Proliferation



(B) IFNγ Secretion

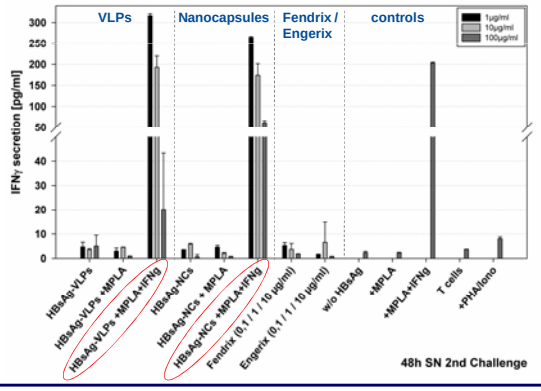


Figure 5: Autologous T cell proliferation and IFNγ secretion after HBsAg-pulsing and maturation of moDCs. (A) Cord blood moDCs were incubated with different doses of HBsAg for 3h and matured with MPLA +/- IFNγ for additional 24h. After HBsAg-pulsing and maturation moDCs were cocultured with autologous T cells for 6 days. 3H-thymidine incorporation was used for the analysis of T cell proliferation. (B) 48h after coculturing of HBsAg-pulsed moDCs with T cells supernatant was used for the investigation of IFNγ levels.

Methods

HBsAg expression, purification and HBsAg-NCs synthesis. Recombinant hepatitis B surface antigen (HBsAg) was expressed with a C-terminal 6xHis-Tag and c-myc epitope using the yeast expression system *Pichia pastoris*. For intracellular HBsAg production yeast cells were grown for 2 days in BMGY medium following 6 days of culture in BMMY medium containing 1% methanol. After protein expression cells were harvested and disrupted in lysis buffer using glass beads. The resulting cell lysate was further purified by Ni-NTA affinity chromatography. HBsAg was used for the synthesis of nanocapsules by the miniemulsion droplet's interface reaction.

Generation, stimulation and maturation of monocyte-derived dendritic cells (moDCs) cocultivated with autologous T cells. PBMCs were isolated from fresh neonatal cord blood by density gradient centrifugtation. $CD14^+$ monocytes were isolated using CD14 MicroBeads and cultured at a concentration of 10^6 cells/ml in 6 well plates in X-Vivo 15 medium (Lonza). Finally, GM-CSF (200 U/ml) and IL-4 (200 U/ml) was added to the medium following 6 days of culture at 37 ºC and 5 % CO_2 in order to generate moDCs. Autologous T cells, selected out of $CD14^-$ cells with the Pan T Cell Isolation Kit (Miltenyi), were cocultured two times for 6 days with moDCs after loading with various concentrations of HBsAg-VLPs or -NCs and maturation with MPLA (4 µg/ml) and IFNγ (50 ng/ml).

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