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Poster for you

Rational immunotherapy design for clinical development against HDV using a synthetic DNA-prime and protein-boost strategy

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Introduction/Summary

- Chronic HDV infection is the most pathogenic viral infection of the liver.
- We have developed a novel DNA-prime/protein-boost immunotherapy that can protect against HBV/HDV co-infection and against HDV-superinfection in uPA/NOD-Scid mice repopulated with human liver cells (1, 2).
- We here describe a rational way of producing a synthetic DNA and a recombinant protein that allows for GMP production at an industrial scale.
- We here show that the synthetic DNA vaccine and the optimized *E. coli* production of the recombinant protein is highly immunogenic.
- We are now ready for clinical development of this new and completely novel therapy against chronic HDV infection.

Study Design

- We wanted to evaluate whether a synthetic DNA vaccine was equally, or more, immunogenic as compared to a bacterially produced plasmid expressing the same PreS1-HDAg sequence.
- A total of seven groups of C57BL/6 mice were immunized, pDNA 50 or 5 µg followed by two protein boosts, dbDNA 50, 32 (equimolar), or 5 µg followed by two protein boosts, pDNA single dose and dbDNA single dose (**Table 1**).
- We wanted to determine if an optimized production protocol in *E.coli* of the protein component, O6N, generated a highly immunogenic protein vaccine.
- The immunogenicity was determined as antibody levels to PreS1 and T cell responses to PreS1 and HDAg.

Table 1: Description of the study design and the study groups

Group	Prime	Dose (ug)	1st Boost	Dose (ug)	2nd Boost	Dose (ug)
1	pDNA-D4	50	O6N + QS-21	20	O6N + QS-21	20
2	pDNA-D4	5	O6N + QS-21	20	O6N + QS-21	20
3	dbDNA-D4	50	O6N + QS-21	20	O6N + QS-21	20
4	dbDNA-D4	5	O6N + QS-21	20	O6N + QS-21	20
5	dbDNA-D4	31,5	O6N + QS-21	20	O6N + QS-21	20
6	pDNA-D4	50				
7	dbDNA-D4	31,5				

Table 1. DNA was delivered *i.m.* followed by *in vivo* electroporation, dose volume of 50uL. Protein was delivered *s.c.*, dose volume of 100uL

Methods

- Our original vaccine designs were based on plasmid DNA encoding multiple preS1 and HDAg sequences and a polypeptide incorporating the same sequences that was unsuited for large-scale manufacture
- pDNA was manufactured in *E. coli* using standard methods, and a synthetic DNA containing the same sequence (doggy-bone DNA, dbDNA) was manufactured enzymatically (Touchlight, UK).
- Protein expression studies were performed in E. *coli*. Protein purity was assessed using standard chromatographic and electrophoretic methods and by reverse phase ultra-performance liquid chromatography combined with mass spectrometry.
- Immunogenicity studies were performed in C57BL/6 mice and analyzed by ELISA and ELISpot assays. (1,2).

Figure 1: Western Blot analysis of pDNA and dbDNA in HEK293 cells

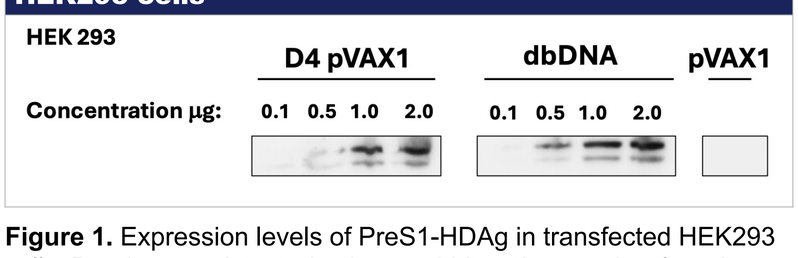

Figure 1. Expression levels of PreS1-HDAg in transfected HEK293 cells. Bands were detected using a rabbit antiserum developed against the pDNA prime/protein boost regimen with the PreS1-HDAg gene

Results

- The dbDNA could effectively express PreS1-HDAg in HEK293 cells (**Figure 1**).
- The protein polypeptide could only be expressed with yields of <1mg/L. Based on the crystal structure of HDAg, each of the four HDAg sequences were expressed separately as an HDAg-preS1 fusion protein from a single operon, resulting in expression at >2g/L.
- Immunoprecipitation, size exclusion chromatography, and UPLC-MS analysis showed that the resulting 29kDa monomers are expressed intact and hetero-oligomerize into a single protein with an apparent molecular weight of >600kDa.
- To facilitate clinical development, we also evaluated the use of dbDNA as the priming component in the immunotherapy, since the enzymatic production is more cost-effective and quicker, and the use of dbDNA means that no selectable marker (antibiotic resistance gene) is needed.
- The new oligomeric protein was fully immunogenic as part of a prime-boost immunization with pDNA when adjuvanted with QS21. Subsequent comparison of the immunogenicity of dbDNA and pDNA, either alone or followed by boosting with adjuvanted oligomeric protein, showed that the dbDNA was fully comparable to bacterially fermented pDNA (**Figure 2**).

Figure 2. PreS1 antibody levels

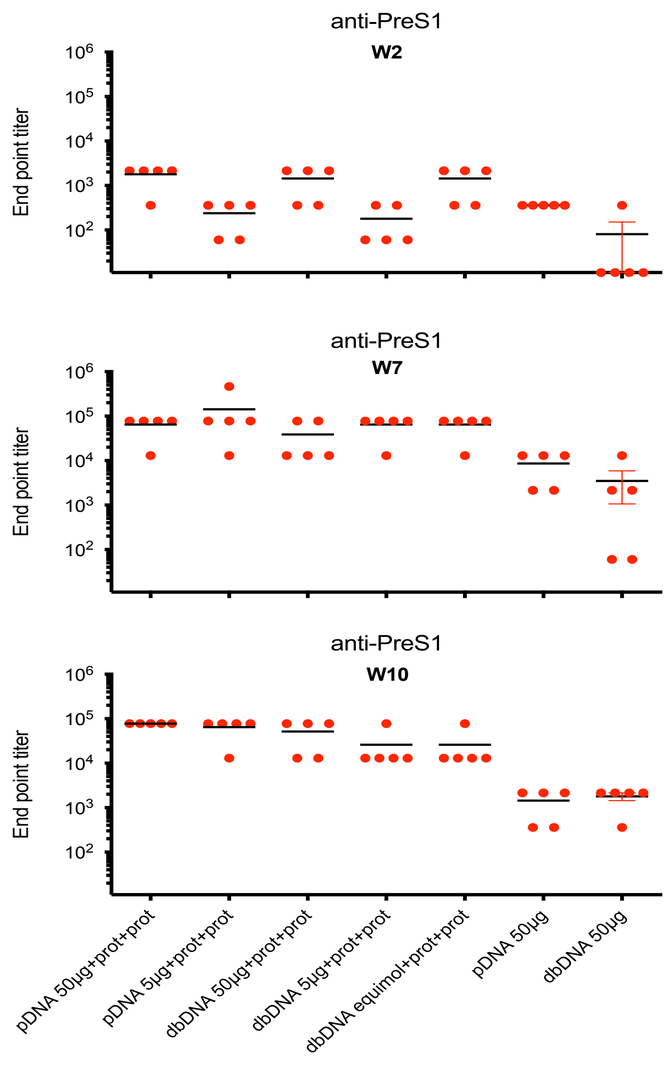

Figure 2. PreS1 antibody levels in C57BL/6 mice after one (W2), two (W7), or three (W10) doses of the PreS1-HDAg vaccines. Mice were immunized on weeks 0, 5 and 8. PreS1 antibodies were detected by ELISA as described (1,2)

- The PreS1 antibody levels averaged from 10^4-10^5 after DNA prime and one or two protein booster doses irrespective of the DNA production method (**Figure 2**).
- Interestingly, the PreS1-specific T cell responses were maintained at a high level using a 10-fold lower dose of dbDNA as compared to pDNA (**Figure 3**).

Figure 3. PreS1- and HDAg-specific T cell responses determined by IFNγ ELISpot

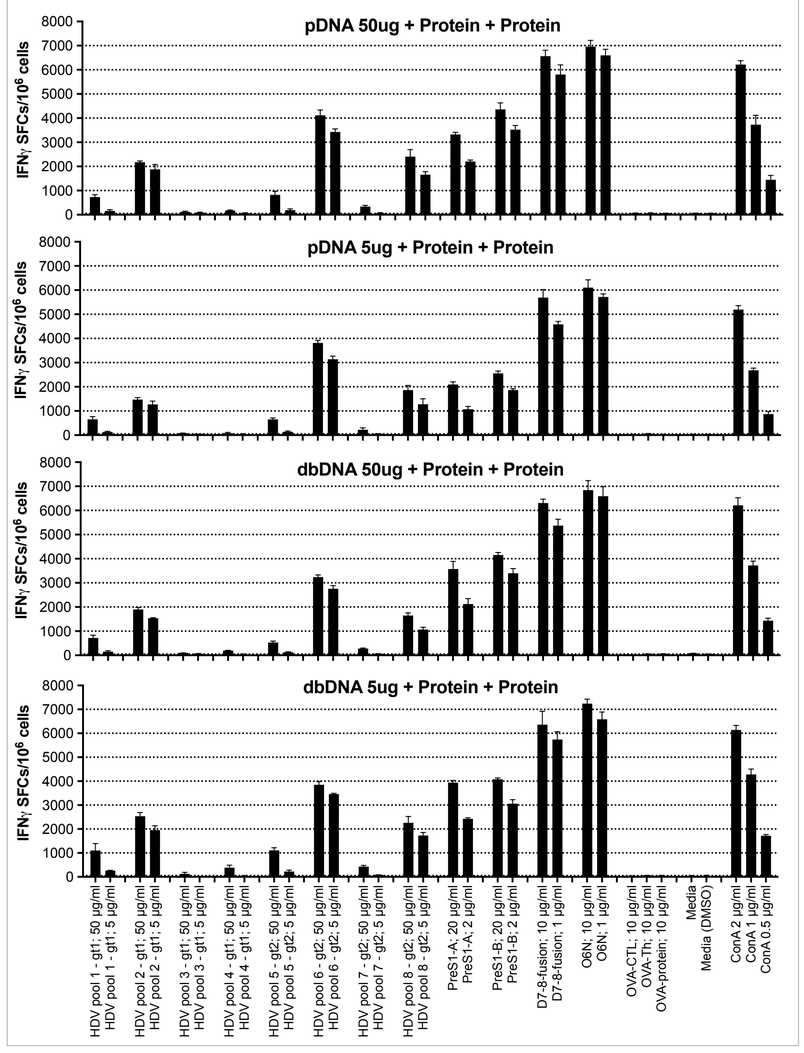

Figure 3. T cell responses primed by either 5 or 50 µg plasmid DNA (upper panel) or synthetic dbDNA (lower panel) were detected by IFNγ ELISpot assay as described (1,2). In brief, mice were immunized as described and two weeks after the last immunization the spleens were harvested and cells were incubated with the indicated antigens for 36 hours. Data has been given as the IFNγ forming spots per million splenocytes.

Conclusions

- A key challenge in clinical development is the ability to produce the drug product(s). We therefore performed preclinical development to ensure that the two active ingredients could be produced according to GMP like protocols. Importantly, this was the case and both components could be produced by the tested approaches.
- This new immunotherapy for chronic HDV infections comprises one or more doses of synthetic DNA encoding PreS1 and HDAg, followed by one or more doses an adjuvanted oligomeric PreS1-HDAg fusion protein produced in *E.coli*. The two components of the vaccine are ready for manufacturing of lots for GLP safety studies and a Phase 1 clinical study.
- This new therapy adds two completely unique properties to the therapeutic arsenal against chronic HDV infection. First, high levels of polyclonal anti-PreS1 antibodies that block HDV infection of cells. Second, T cells that support PreS1 antibody production and eliminate HDAg-expressing cells.
- To summarize, we are now able to enter clinical development of this new and unique therapy against chronic HDV infections.

Contact information

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References

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