Enhanced Immune Response To Vaccination With Woodchuck Hepatitis Virus (WHV) Surface Antigen (WHsAg) Using Cationic Liposome-DNA Complexes (CLDC) As Adjuvant

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Background

Immunity to hepatitis B virus (HBV) involves efficient antiviral B and T cell responses during the acute phase of HBV infection leading to resolution of viral infection. Humoral and cellular immunity to HBV is also achieved following prophylactic vaccination of healthy individuals with conventional vaccines that are based on the HBV envelope protein and alum-adjuvant. Contrary in patients with chronic HBV infection, persistent viral replication is associated with deficient B and T cell responses to HBV proteins. Recovery of both cell activities in chronic HBV carriers by therapeutic vaccination appears a promising approach for viral eradication.

Complexes of cationic lipid carrier and non-coding plasmid DNA (CLDC) are a potent stimulant of innate immunity. Stimulation by CLDC is mainly due to a liposome-mediated potentiation of the inherent responsiveness of the mammalian immune system to non-methylated CpG motifs within the bacterial plasmid DNA. CpG motifs function *via* interaction with the toll-like receptor 9, an interaction that requires internalization facilitated by the lipid component. CLDC can be combined with specific bacterial or viral antigens to produce pathogen-specific vaccines. These lipid-DNA-antigen complexes result in a potent adjuvant effect with elicitation of robust antibody and cell-mediated immune responses to the target antigen.

Objective

Using the woodchuck animal model of HBV infection the immunogenic effects induced by CLDC were investigated during prophylactic vaccination of woodchuck hepatitis virus- (WHV) negative woodchucks with three doses of WHV surface antigen (WHsAg) adjuvanted with either CLDC or alum and administered intramuscularly (IM) or subcutaneously (SC) (Figure 1).

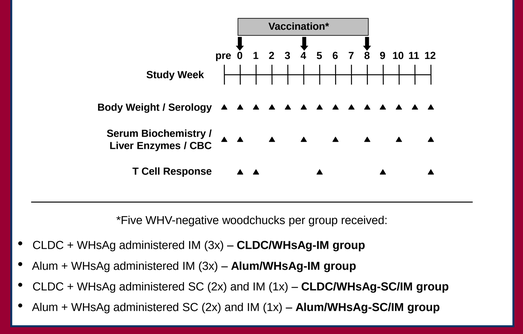
Results

Antibody response: IM vaccination with CLDC/WHsAg elicited anti-WHs earlier and in more woodchucks than did Alum/WHsAg, with significant different titers at wk 3 (Figure 2). Overall, titers were greater and antibody responses more sustained with CLDC/WHsAg than with Alum/WHsAg (Figure 2, Table 1). Because antibody responses at wk 8 were 2.7 to 9.9-fold higher in the IM than in the SC groups, woodchucks given SC vaccine at wks 0 and 4 received the wk 8 vaccine IM. Antibody responses increased subsequently to titers similar or higher than those in woodchucks given CLDC/WHsAg by the IM route (Figure 2, Table 1) .

T cell responses: Differences in T cell responses to WHsAg and selected WHsAg peptides between groups were detected at wk 5 (Figures 3&4): Three of 5 woodchucks from the CLDC/WHsAg-IM group had T cell responses to WHsAg and most peptides were recognized In contrast, only 1 of 5 woodchucks from the Alum/WHsAg-IM and Alum/WHsAg-SC/IM groups (none in the CLDC/WHsAg-SC/IM group) had T cell responses to WHsAg that were weaker and recognized fewer peptides. After the third immunization, T cell responses were similar in all vaccinated groups but were more sustained in the CLDC/WHsAg group (Figure 5).

Conclusions

- CLDC-adjuvanted WHsAg administered IM results in a more rapid enhancement of humoral and cellular immune responses compared to a conventional alum-adjuvanted vaccine.
- While less rapid, the responses following SC administration of vaccine can prime the IM responses.
- The enhanced adjuvant activity of CLDC over alum could be beneficial for therapeutic vaccination in chronic HBV infection which is currently studied in woodchucks with chronic WHV infection.



*Five WHV-negative woodchucks per group received:

- CLDC + WHsAg administered IM (3x) – **CLDC/WHsAg-IM group**
- Alum + WHsAg administered IM (3x) – **Alum/WHsAg-IM group**
- CLDC + WHsAg administered SC (2x) and IM (1x) – **CLDC/WHsAg-SC/IM group**
- Alum + WHsAg administered SC (2x) and IM (1x) – **Alum/WHsAg-SC/IM group**

Figure 1. Study design.

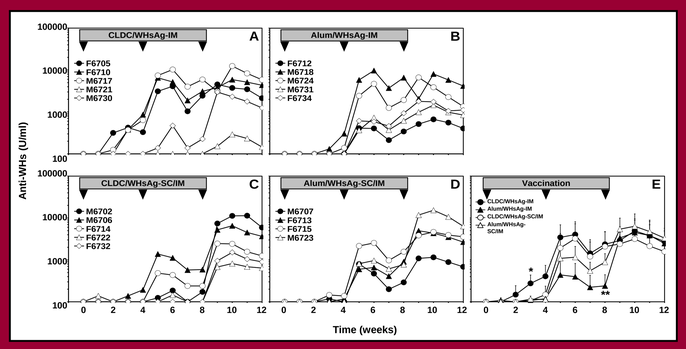


Figure 2. Serum antibody responses. *, Mean anti-WHs titer for CLDC/WHsAg-IM group was statistically different from Alum/WHsAg-IM and CLDC/WHsAg-SC/IM groups at wk 3 ($P < 0.05$); **, Mean anti-WHs titer for Alum/WHsAg-SC/IM group was statistically different from CLDC/WHsAg-SC/IM group at wk 8 ($P < 0.05$).

Anti-WHs titers and responder rates and sample frequencies with positive anti-WHs following vaccination of WHV-negative woodchucks with CLDC-adjuvanted WHsAg vaccine or alum-adjuvanted WHsAg vaccine administered IM or SC/IM.

Group	Mean anti-WHs titers (U/ml [± SD])		Cumulative group responder rate (CGRR) (%)		Cumulative sample response frequency (CSRF) (%)	
	Following 1st dose (week 4)	Following 3rd dose (week 10)	Following 1st dose (week 4)	Following 3rd dose (week 12)	Following 1st dose (week 4)	Following 3rd dose (week 12)
CLDC/WHsAg-IM	**406** (± 337)	4932 (± 4658)	**60**	100	**40**	73
Alum/WHsAg-IM	161 (± 88)	3521 (± 2990)	40	100	15	72
CLDC/WHsAg-SC/IM	119 (± 42)	4506 (± 4374)	20	100	15	58
Alum/WHsAg-SC/IM	112 (± 18)	6364 (± 6178)	50	100	25	75

Table 1. Mean anti-WHs titers detected following the first (wk 4) or the third dose of vaccine (wk 10, maximum titer in all groups) are presented. CGRR (= woodchucks with positive anti-WHs/total number of woodchucks × 100) and CSRF (= number of samples with positive anti-WHs/total number of samples × 100) were calculated following the first (wks 1 to 4) or the third dose of vaccine (wks 1 to 12).

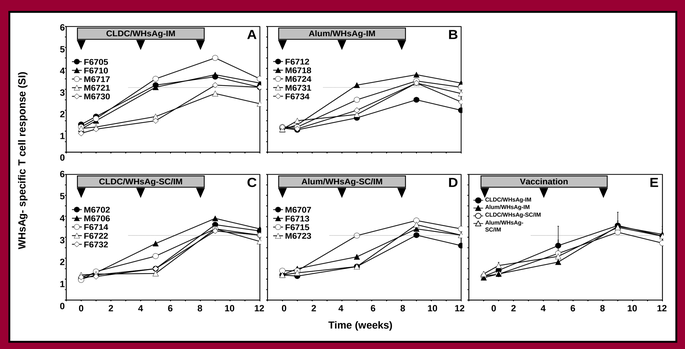


Figure 3. T cell responses to WHsAg. T cell response was positive, if SI was ≥ 3.1 as indicated by the dotted line.

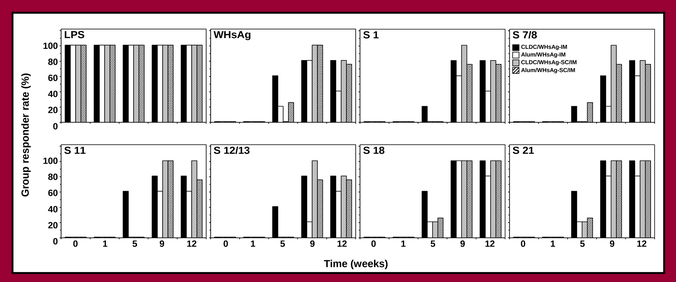


Figure 4. Group responder rates for positive T cell responses to WHsAg and selected WHsAg peptides for each time point of the study. For comparison, the group responder rates to the polyclonal activator lipopolysaccharide (LPS) used as a positive control is shown.

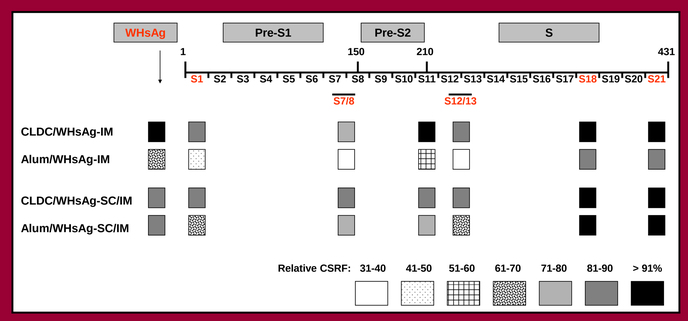


Figure 5. Comparison of WHs-specific T cell responses. CSRF (= number of samples with positive T cell response/total number of samples × 100) were calculated following the first dose of vaccine (wks 1 to 12) and converted to relative values within ranges for each group. Relative CSRFs were calculated by setting the observed CSRF to WHsAg at 100% for the CLDC/WHsAg-IM group.