Summary

The recently concluded human phase II clinical trials of the contraceptive vaccine based on the heterospecies dimer of βhCG non covalently associated with αoLH and coupled to carrier proteins such as TT or DT (HSD vaccine) has demonstrated the efficacy of anti-hCG antibodies in preventing pregnancy in immunized subjects. The main objective of this study was to delineate epitope specific antibody responses in humans against hCG. The subjects immunized with HSD vaccine offered an excellent model system for such an investigation. Such studies had not been reported earlier and no information was available with respect to anti-hCG antibody repertoire in women. Two approaches were used to delineate epitope specific antibody responses against hCG in women immunized with the HSD vaccine. Either monoclonal antibodies were used as epitope specific probes and inhibition EIAs developed or synthetic peptides representing different epitopic domains of hCG were employed. The salient findings of this study are:

1) A panel of anti-hCG MAbs was developed and characterized. Four MAbs representative of different epitopic domains on hCG were employed to develop inhibition EIAs for analysis of epitope specific antibody responses in women immunized with the HSD vaccine.

2) A preponderance of antibodies against an epitopic domain defined by the MAb 206 was seen in random bleeds of all subjects. This phenomenon was also observed in the sequential bleeds of 5 representative subjects throughout the immunization schedule.

3) The antibody titer against the epitopic domain defined by MAb 206 (designated as ED_{50}) had a good correlation with the in vitro bioneutralization capacity of the sera (r = 0.75, p < 0.001). Analysis of
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sequential bleeds showed that the ED$_{50}$ value follows the bioneutralization capacity throughout the immunization schedule.

4) Their was a lack of antibody response against one of the receptor binding domain of βhCG represented by the βhCG loop peptide 38-57. A MAb 357-2 directed against this epitopic domain failed to inhibit the binding of human anti-hCG antibodies to hCG. Moreover, none of the serum samples tested, recognized the βhCG loop peptide 38-57 in a direct binding ELISA.

5) Antibody response against the CTP of βhCG (109-145), which forms the basis of another vaccine was also analyzed. CTP was poorly immunogenic in women, of the 28 subjects analyzed only 57% had detectable anti-CTP antibody response. Moreover the anti-CTP titer had no correlation with the total anti-hCG antibody titres. Our studies demonstrate for the first time that contraception can be achieved in HSD immunized women despite lack of anti-CTP antibodies.

6) Anti-hCG antibody repertoire was also studied in rats where hCG is a foreign antigen. MAb 206 defined epitopic domain was immunogenic in all rats suggesting promiscuous nature of this epitopic domain. As in humans the epitopic domain represented by the βhCG loop peptide 38-57 was poorly immunogenic. However, in contrast to humans, the CTP was highly immunogenic in rats. All rats had made antibodies against this domain. Moreover, there was a good correlation between the anti-CTP and anti-hCG antibody titres.

7) The epitopic domain defined by MAb 206 is exposed on the hCG-receptor complex. The in vitro bioneutralization capacity of MAbs recognizing overlapping/similar epitopes within this epitopic domain was
a function of their affinity. However, this phenomenon could not be confirmed \textit{in vivo}.

8) The epitopes within the domain defined by MAb 206 are conformational or discontinuous ones as none of the antibodies recognized the RCM-\textbeta hCG. This epitopic domain is probably defined by amino acids 1-40 and 60-105 of \textbeta hCG.

9) IgG subclass determination of anti-hCG antibodies showed that the response was predominantly of IgG1 subclass, with a variable IgG4, poor IgG2 and undetectable IgG3. The \textit{in vitro} bioneutralization capacity of sera from subjects not having IgG4 antibodies was not significantly different from those having antibodies of IgG4 subclass. Similar to hCG, the anti-DT antibody response was predominantly of IgG1 subclass.

In conclusion these studies have highlighted the quality of antibody response generated against hCG in women immunized with hCG based vaccines for the first time. These studies would help in the development of rationale synthetic immunocontraceptive vaccines based on hCG.