Construct, design and immunogenicity of a minicircle DNA vector expressing a small HIV-peptide

Sofia Stenler*, Lena Hansen**, Jorma Hinkula***, Stefan Petkov**, Karl Ljungberg**, Karin E Lundin****, Pontus Blomberg*, Britta Wahren**
*) Vecura, Karolinska University Hospital, Sweden **) MTC, Karolinska Institute, Sweden ***) IKE, Linköping University, Sweden ****) LabMed, Karolinska Institute, Sweden

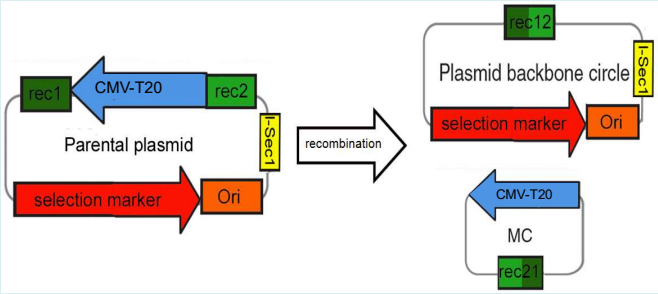

Figure 1: The minicirle production system
Induction of recombination of the parental plasmid at the sites flanking the expression cassette results in the MC and a separate construct containing the bacterial backbone.

rec1, rec2: Recombination sites; CMV-T20: T20 expression cassette; Ori: origin of replication; rec12, rec21: post-recombination site-hybrids, I-Sec1: rare restriction enzyme recognition site

Novel DNA vaccine vectors for short peptides

We explore different constructs to deliver a DNA encoding a short immunogenic HIV-1 peptide. We have used the plasmid based minicircle vector (MC), devoid of bacterial sequences, and compare immunogenicity to a conventional plasmid vector as well as to the peptide.

We demonstrate a clear-cut induction of antibodies to the T20 peptide that competes with binding of the 2F5 antibody. MC together with plasmid HIV-DNA strongly enhanced endpoint titers against both gp140 C and T20 compared to HIV-DNA alone. Together with HIV-DNA, plasmid and MC but not T20 peptide elicited T20-specific IFN-γ responses.

Background

The MC is known for its prolonged expression and increased robustness as compared to conventional DNA plasmids.

The peptide expressed by the MC, T20 Fuzeon Enfuvirtide, is a well-established antiviral drug against HIV. As T20 blocks virus entry into CD4+ cells by mimicking part of the viral transmembrane protein, it might also serve as an antigen of HIV. The T20 expressed by the MC contains the epitope for a broadly neutralizing antibody, 2F5.

Cloning and production

A novel T20 MC construct was cloned, encoding the 108 base pairs (bp) long sequence of T20 flanked by the CMV promoter and other regulatory sequences. The entire MC encompasses 1 134 bp.

The MC was produced in *E.Coli*, by recombination of the parental plasmid into a MC containing only the expression cassette and the backbone containing Ori and antibiotics resistance. (see Figure 1 above).

Expression

Expression of peptides as short as 36 aminoacids is not trivial, in nature they are often produced as multimers which are then cleaved.

Thus we first tested expression *in vitro,* and through western blotting we could confirm the peptide production from the MC.

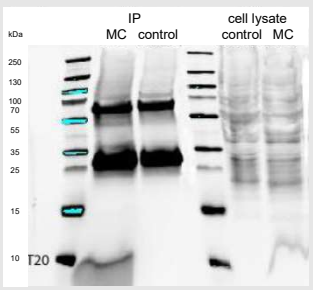

Figure 2: Wester blot analysis of in vitro expression of T20 in immunoprecipitated samples (IP) and in cell lysate.
MC: MC transfected cells
Control: untransfected cells

Immunogenicity

Mice were immunized intradermally by electroporation with MC alone, or MC or plasmid (P) in combination with HIV-DNA-Env plasmids encoding Env subtypes A, B, C (pHIVDNA).

Antibody response

The MC-T20 alone elicited a T20 specific antibody response. Mice immunized with HIV-DNA-ENV alone had significantly lower end point titers against both gp140 C and T20 than when the HIV-DNA-Env had been given together with MC-T20. Sera from mice immunized with HIV-DNA-Env plasmids and the T20 plasmid resulted in increased antibody titers also against other sites of the gp160 HIV protein. The majority of these latter antibodies were not T20-specific.

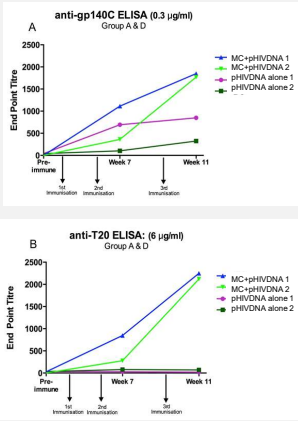

Figure 3: Binding antibodies to subtype C gp140 (A) and T20 (B) in high responders.

Cellular response

Spleenocyte analysis showed T20-specific cellular responses for the mice receiving the MC or the plasmid as adjuvants. Neither the peptide nor the full length HIV-DNA-Env plasmids alone elicited any response.

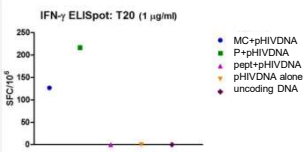

Figure 4: Splenocytes from pooled groups were stimulated with T20 antigen and IFN-γ responses were measured.

Summary

Minicircle vectorized T20 elicits a potent HIV T20 antibody response, and in combination with HIV Env plasmids it enhances production of T20 and gp140 binding antibodies.

Dr Stenler has a PhD from Karolinska Institutet and is currently looking for a postdoc position in DNA vaccination.

Karolinska Institutet
Sofia Stenler • PhD

E-mail: sofia.stenler@gmail.com
Telephone: +46(0)70-457 89 54

Read my thesis:

https://goo.gl/8ysfvd

Support by
Vactrain
and
Karolinska Institutet

KAROLINSKA INSTITUTET ANNO 1810

Karolinska Institutet