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A DNA HIV-1 vaccine based on a fusion gene expressing non-structural and structural genes of consensus sequence of the A–C subtypes and the ancestor sequence of the F–H subtypes. Preclinical and clinical studies

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Abstract

A potent DNA vaccine against HIV, combining a vector that takes advantage of the segregation and compartmentalization effect of bovine papilloma virus E2 protein with MultiHIV insert, expressing a fusion gene coding for the non-structural and structural proteins was developed and tested for immunogenicity in mice and humans.

Keywords: HIV vaccine; MultiHIV

1. Introduction

The task of eliciting a protective immunity towards HIV faces major problems including the variability of incoming viruses and local immunomodulation induced by HIV-infected cells. The spread of the virus after exposure is primarily by direct cell-to-cell contact, and thus, HIV infection is predominantly transmitted through infected cells. The major protective mechanism seems to be a strong and broad T-cell immunity, able to recognize and kill the virus-infected cells. Because of the polymorphism of the human histocompatibility system, and the variability of the virus, an effective HIV vaccine should contain as many HIV genes/proteins as possible, including structural, regulatory and auxiliary genes/proteins. The cellular responses raised against these proteins could overcome the down regulation of major histocompatibility complex (MHC) and CD4 by HIV itself, the mechanism which protects virus-infected cells against the lytic effects of natural killer and T-cells.

To gather the majority of the T-cell epitopes in one vaccine construct, we have developed a multigene/multi-epitope vaccine and tested it in preclinical and clinical trials. As a vector, we have developed a specific type of DNA plasmid, named Gene Transport Unit (GTU®), that takes advantage of the special feature of human papilloma virus maintenance and segregation in dividing cell populations. Such a DNA vaccine has shown strong immunogenicity, as well as protective capacity in preclinical animal experiments and challenge model.

2. Generation of the vector and vaccine construct

2.1. Generation of the GTU® and auxo-GTU® vector constructs

A DNA vector, termed GTU® with enhanced expression capacity has been developed and used to immunize HIV-

Abbreviations: BPV, bovine papilloma virus; CMV, cytomegalovirus; CTL, cytotoxic T lymphocyte; EP, electroporation; g.g., gene gun; GTU®, Gene Transport Unit; HAART, highly active anti-retroviral therapy; i.d., intradermal; IFN-γ, interferon-gamma; i.m., intramuscular; LPA, lymphocyte proliferation assay; MHC, major histocompatibility complex.

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infected and non-infected volunteers with the *nef* gene or with a fusion gene expressing several HIV-1 regulatory and structural genes. The main feature of GTU® is its capacity for long maintenance and high expression in both dividing and non-dividing cell populations. The GTU® takes advantage of the viral partitioning and transcriptional activation function of the E2 protein from the bovine papilloma virus (BPV), thus leading to high and prolonged expression of the gene of interest [1].

A composition of the GTU® vector is presented below (Fig. 1).

In addition to the standard components of eukaryotic expression vectors, such as expression cassette (or cassettes) for a gene of interest driven by promoter P1, usually strong cytomegalovirus (CMV) promoter and the intron/poly A sequence, the GTU® carries an additional expression cassette for E2 with an other viral promoter (like RSV LTR or a CMV promoter). Furthermore, multimeric E2 protein binding sites, usually 10 copies of BPV1 binding site 9, have been added to the GTU® vector. The plasmid is produced in *Escherichia coli* using *ColE1* origin for replication and antibiotic selection markers for kanamycin or ampicillin, or encoding for the enzyme assuring complementation of the auxotrophic defect of bacterial strain that has been used in production process.

To demonstrate the segregation function of GTU®, we developed a GTU®-d1EGFP expression vector allowing to identify the number of cells with transcriptionally active plasmid and the level of expression per cell using EGFP as a reporter protein detection by FACS. This allowed testing the maintenance of the plasmid in dividing cells, as compared to the regular CMV vector by flow cytometry counting the plasmid positive EGFP expressing cells in transfected cells. The results are presented below (Fig. 2).

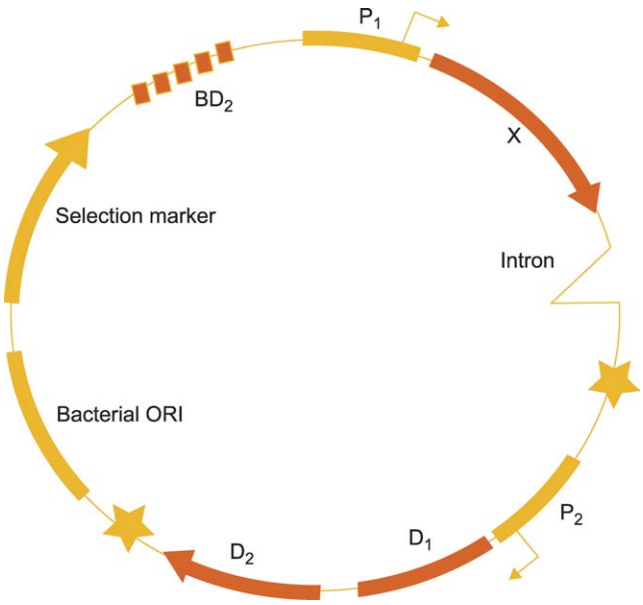

Fig. 1. Schematic presentation of the GTU® plasmid vector. P1 promoter of the gene of interest. X = coding sequence of the gene of interest. P2 promoter for the coding sequence of DNA/nuclear compartment binding protein D1–D2. ★ polyadenylation signals. D1 domain for attachment to the nuclear compartment. D2 domain for DNA binding BD2 D2 binding sites.

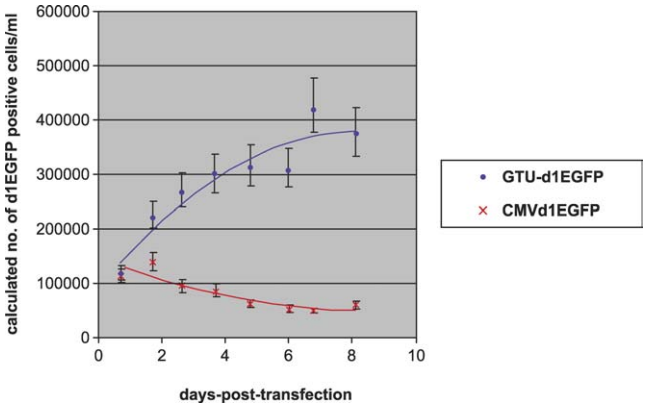

Fig. 2. Maintenance of constructed plasmid in dividing cells in comparison with CMV vectors.

As can be seen from the Fig. 2, GTU® functions fundamentally in a different way, as compared to the ordinary expression vectors. The number of GFP positive cells is increasing in time indeed, as it was predicted, and on day 8 there are at least six times more plasmid positive cells in the case of GTU®, as compared to the regular CMV vector. Depending on the cells and the experimental conditions, the plasmid positive cell number may increase 50 times after 2 weeks of cultivation without any replication of the vector.

According to the paradigm, the spreading of these GTU® vectors is due to the multimeric E2 high affinity binding sites and to the presence of the E2 protein, which assure the attachment of the vector to the mitotic chromatin (Fig. 3).

The variant of GTU®, Auxo-GTU®, can be produced in bacterial cells, without an antibiotic selection system. The antibiotic resistance gene has been replaced with the Ara D gene, which enables the growth of the plasmid in Ara D deficient mutated *E. coli* strains without the use of antibiotics in the presence of an alternative carbon source and selection agent arabinose instead of glucose (Toots et al., manuscript in preparation).

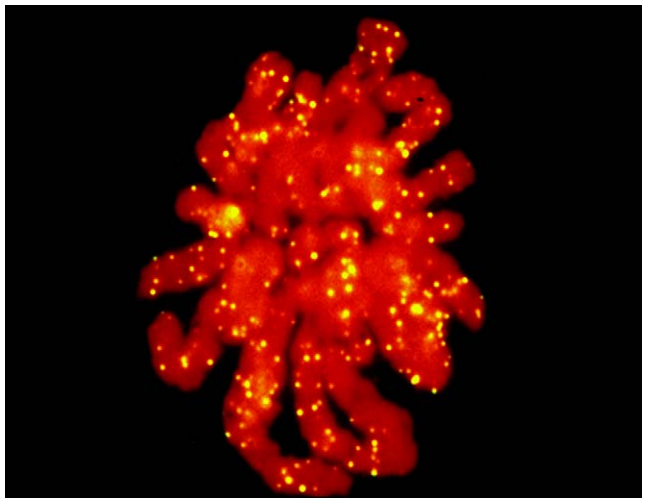

Fig. 3. Chromatin attachment. E2 protein mediates the tethering of the E2 binding sites of the plasmids to the metaphase chromosome.