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Short communication

A multivalent HIV-vaccine: development of a plasmid DNA for the expression of HIV envelope glycoproteins with hypervariable V3-loop domains

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Abstract

The third hypervariable (V3) loop of the HIV-1 envelope glycoprotein gp120 plays an essential role in the process of viral entry. It contributes to the tropism, coreceptor usage and immune-escape of the virus. We generated a monovalent plasmid DNA and demonstrated the expression of HIV-1 clade B subtype NL4-3 gp120 and gp160 in comparison to a multivalent plasmid DNA encoding for a variety of V3-variants. In contrast to the membrane-anchored gp160, preliminary data demonstrate the monovariant gp120 is expressed in and presented on a human dendritic cell (DC) line, due to a HIV*env*-specific re-stimulation of naïve T-cells detected by IFNγ-ELISPOT assay.

Keywords: HIV; DC; gp120; V3; Immune escape; Variability

1. Introduction

The human immunodeficiency virus (HIV) envelope glycoprotein mediates receptor/co-receptor binding and is the major target for neutralising antibodies. As a consequence to avoid clearance, HIV acquires mutations particularly in the V3-loop region of the envelope glycoprotein to generate immune-escape variants, which are resistant to neutralisation [1]. In addition to amino acid exchanges altering the antigenic profile, conformational modification of the envelope protein due to N-linked glycosylation is also utilised by the virus to escape the immune system [2].

2. Development of a hypervariable HIV-V3 plasmid DNA vaccine

In this study, we present data for the development of a multivalent DNA-vaccine, based on a large range of defined variations within the HIV envelope glycoprotein. Thus, we have developed a plasmid DNA which contains a HIV-1 clade B NL4-3-type rev/env gene cluster, encoding for full-length gp160 envelope protein. Based on this plasmid DNA, a second construct was developed by a single-nucleotide exchange at the furin cleavage site to introduce a stop-codon for the expression of secreted gp120 envelope protein. In parallel, a multivalent HIV-1 clade B MN-type V3-bank was generated by PCR using random amplification of different combinations of distinct V3-olignucleotides to produce a variety in the order of 10^4 different V3-loop regions, which were inserted into the C2/V3-loop region of the HIV*env* gene (Fig. 1). Consequently, this hypervariable DNA vaccine generates modifications within the antigenic shape of the HIV envelope protein in combination with alterations of its glycosylation motifs.

3. Characterisation of the DNA vaccine

The DNA-vaccine was analysed as single envV3-clones or as the entire envV3-library by in vitro expression stud-

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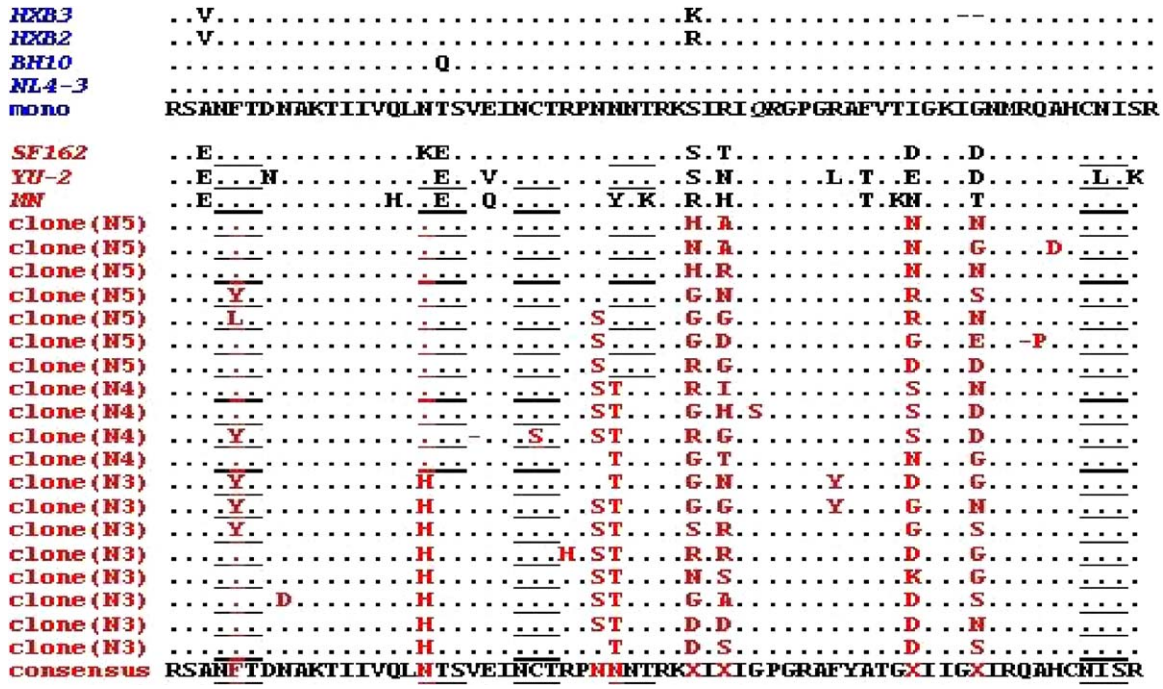

Fig. 1. Sequence analysis of monovariant DNA vaccine (blue) and individual clones of the multivariant HIV vaccine (red). As a reference, related C2/V3 regions of established HIV genomes are added. The amino acid sequences of the HIVenv C2/V3 region are displayed with exchanges highlighted and potential glycosylation motifs underlined. The number of *n*-glycans per clone are presented (*n*3–*n*5). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

ies using several human cell lines. As expected, the results demonstrated individual differences between the multivariants and a corresponding monovariant with respect to the antigenic profile by anti-gp120 ELISA (data not shown). The comparison of a monovariant V3-loop gp120 protein with the envV3-library containing various V3-loop amino acid changes showed differences by using a polyclonal anti-gp120 antibody for immunoprecipitation, due to the amino acid diversity flanking the immunodominant V3-epitope and irrespective on changes of the glycosylation arrangement. Moreover, although the gp160 envelope glycoprotein was located primarily in the cellular fraction, the multivariants were secreted solely into the culture medium (Fig. 2), indicating the desired effect for an envelope protein that is released in the periphery. As expected, the gp120 envelope protein,

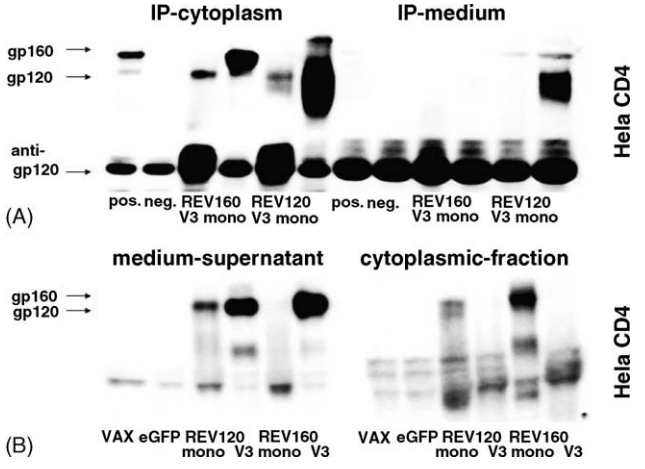

Fig. 2. Immunoblot analysis of the HIV vaccine expression in transiently transfected HeLa CD4+ cells. (A) Immunoprecipitation (IP) using a polyclonal anti-gp120 of medium and cellular fractions. (B) Immunoblot of extracts from medium and cellular fractions. Signal for gp120 and gp160 envelope proteins are indicated by arrows. Legend: VAX = neg.Ctrl.; REV160 = full-length HIV*env*; REV120 = truncated HIV*env*; mono = monovariant DNA vaccine; V3 = multivalent DNA vaccine.

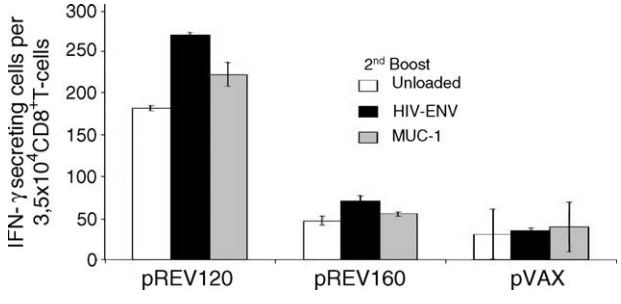

Fig. 3. CD8+ T-cell activation by dendritic cells nucleofected with an HIV vaccine. Nucleofected mDCs and naïve CD8+ T cells are mixed in a 1:10 ratio. The surviving CD8+ T cells are restimulated to test for specific recognition of the envelope-related peptide (HIV-ENV) encoded by both the gp120 and the gp160 vaccine but not the empty PVAX plasmid. The peptide is in vitro loaded onto MHC class I molecules on HLA-A2 positive T2 cells. As negative controls CD8+ T cells are stimulated with an irrelevant MUC-1 peptide as well as non-loaded T2 cells. Thirty-five thousand CD8+ T cells are restimulated with 7000 T2 cells and analysed in an IFN-γ ELISPOT assay, where the number of IFN-γ secreting cells is detected.

which was expressed without the membrane anchor gp41 envelope protein, was secreted into the extracellular compartment, independent of whether this protein was present as monovariant or as multivariant.

4. Immunogenicity of the DNA vaccine

To address the question whether the HIV vaccine was able to induce a T-cell response, a human dendritic cell (DC) line was nucleofected with the plasmid DNA. These immortalised CD34+/CD14+ cells can be differentiated and matured to fully functional immature (iDC) and mature dendritic cells (mDC) to process and present viral antigens in the context of MHCI, MHCII or CD1, thereby potently activating naïve and memory CD8+ and CD4+ T cells and regulatory T cells. Preliminary data demonstrated expression of the monovariant DNA-vaccine in transiently transfected dendritic cells by HIV-specific RT-PCR and immunoblot detection of gp120, clearly indicating envelope protein was produced, processed and presented on MHC class I molecules (data not shown). Further investigation showed naïve T cells can be specifically induced, because CD8+ T cell responses against HIV were observed by IFNγ-ELISPOT analysis after stimulation with an env-specific peptide representing a well-known CTL-epitope (Fig. 3). In addition, the multivalent HIV vaccine has recently been used for an immunisation study in mice to develop antisera, which will be analysed in neutralisation assays and tested for their broad range of detection by individual clones to analyse whether the DNA vaccine was able to induce a humoral immune response.

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