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Construction and characterisation of replicating foamy viral vectors expressing HIV-1 epitopes recognised by broadly neutralising antibodies

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ABSTRACT

With the aim to develop a replicating vector system for the delivery of HIV-1 antigens on the basis of an apathogenic foamy virus we recently showed that immunisation with purified recombinant hybrid antigens composed of the feline foamy virus Bet protein and parts of the transmembrane envelope protein of HIV-1 induced antibodies with an epitope specificity identical to that of the broadly neutralising antibody 2F5 (Mühle et al., Immunol Res., 2013, 56:61–72). Here we set out to further improve the HIV-1 inserts consisting of the membrane proximal external region (MPER) and the fusion peptide proximal region (FPPR) by stepwise shortening distinct linker residues between both domains. In a subset of these antigens, enhanced recognition by 2F5 and 4E10 was observed, indicating that a specific positioning of FPPR and MPER domains is critical for improved antibody binding. Introduction of these optimised inserts as well as of the MPER domain alone into the feline foamy virus backbone was compatible with virus replication, giving viral titres similar to wild-type virus after extended passaging. Most importantly, expression of the HIV-1 transgenes in infected feline CRFK cells remained stable in three out of four constructs and was detectable after serial passages for several weeks. These data encourage further testing of these vectors *in vivo*, which may allow insights into the necessity of affinity maturation for the induction of broadly reactive HIV-1 antibodies.

1. Introduction

Broadly neutralising antibodies (bnAb) directed against the surface envelope protein gp120 and the transmembrane envelope (TM) protein gp41 have been found in about 20% of HIV-1 infected individuals (Burton et al., 2005; Doria-Rose et al., 2009; Mascola and Montefiori, 2010). They were able to prevent infection after passive immunisation (Ferrantelli et al., 2004; Hessell et al., 2009, 2010) and are protective when expressed ectopically after gene transfer (Balazs et al., 2012). A subset of bnAb such as 2F5 (Muster et al., 1993), 4E10 (Stiegler et al., 2001), 10E8 (Huang et al., 2012), and 2H10 (Lutje Hulsik et al., 2013) target the conserved membrane proximal external region (MPER) of gp41 and additional epitopes within MPER are gradually identified (Zhou et al., 2013). Despite being a promising vaccine target, most attempts to induce MPER-directed bnAb by vaccination failed so far (reviewed by Montero et al., 2008). Several of the broadly reactive antibodies including those targeting the MPER show a high degree of somatic mutations, prolonged CDRH3 loops, lipid binding properties or polyreactivity (Burton et al., 2005; Haynes et al., 2012). Furthermore, they arise after extended time of infection (~4 years) and their potency correlates with increased avidity to Env (Gray et al., 2011; Sather et al., 2009). This supports the view that affinity maturation is an essential factor in the development of such highly effective antibodies, which might be only achieved by extended antigen presentation.

In order to establish a system that allows long-term, persistent antigen presentation, we develop replicating vectors based on foamy viruses (FV), and in particular, the feline FV (FFV), to allow evaluation of such novel vectors in cats. Since FV from the *Spumaviriniae* sub-family induce persistent but apparently apathogenic infections, trigger strong humoral immune responses and replicate actively in mucosal tissues, they are exceptionally suited for such purposes (Lindemann and Rethwilm, 2011; Liu et al., 2013; Trobridge, 2009). Their low prevalence in humans (~2% in individuals exposed, e.g., to infected simians) as dead-end host (Callahan et al., 1999; Heneine et al., 2003, 1998) and preferential integration into transcriptional inactive regions (Deyle et al., 2013; Trobridge et al., 2006) makes them furthermore an interesting alternative to existing systems that were abandoned due to issues

Abbreviations: FFV, feline foamy virus; HIV-1, human immunodeficiency virus-1; MPER, membrane proximal external region; FPPR, fusion peptide proximal region.

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of pre-existing immunity or safety concerns. By being immunogenic, highly expressed and secreted from infected cells (Bleiholder et al., 2011; Giron et al., 1998; Lecellier et al., 2002; Lindemann and Rethwilm, 1998; Romen et al., 2006) the accessory Bet protein of FV shows favourable properties as carrier molecule for the induction of humoral immune responses including conformation-sensitive antibodies (Alke et al., 2000).

In our previous work we antigenically and immunologically characterised purified recombinant fusion proteins expressed in *E. coli*, which were composed of the accessory FFV Bet protein and HIV-1 gp41 epitopes (Mühle et al., 2013). The MPER domain alone, or the MPER linked to the N-terminal fusion peptide proximal region (FPPR, HIV-1 HXB2 Env residues 525–546), were appended to the Bet antigen. The latter approach was based on previous reports showing that the presence of FPPR residues enhanced 2F5 binding and stabilised a conformation in MPER characterised by an increased beta-sheet content (de la Arada et al., 2009; Fiebig et al., 2009; Noah et al., 2008). A similar structure has been described for the MPER epitope in a 2F5-bound state (Bryson et al., 2009; Julien et al., 2008; Ofek et al., 2004) and therefore it might represent the initial B-cell trigger for the generation of 2F5-like antibodies. Whereas these antigens elicited antibodies binding to the 2F5 epitope and native Env on the surface of infected cells, enhanced recognition by 2F5 in presence of the FPPR or neutralisation were not observed (Mühle et al., 2013).

Here we attempted to improve the previous immunogen design by testing an extended set of FPPR/MPER loop antigens and analysed the compatibility of the strategy with HIV-1 transgene expression and virus replication when transferred into a replicating FFV.

2. Material and methods

2.1. Prokaryotic expression plasmids

A DNA template plasmid encoding the amino acid sequence of the construct F0-M0 and 11 spacer amino acids (SGSAGAGAGGG), which contained restriction sites for subcloning (Supplementary Fig. 1), was synthesized by GeneArt (Invitrogen, USA). Constructs F0-M1 to F2-M2 were derivatives of this construct obtained by PCR mutation of whole plasmids with combinations of phosphorylated primers (Supplementary Table 1), resulting in single amino acid deletion on the C- or N-terminus. The PCR products were ligated, transformed in *Escherichia coli* and analysed by sequencing. The mutated plasmids were digested with *Mly*I/*Hind*III and the inserts were cloned into the prokaryotic expression vector pQE-30 Xa (Qiagen, Germany), which had been digested by *Stu*I/*Hind*III. Positive clones were sequenced to confirm insert integrity.

2.2. Protein expression and purification

After optimisation of expression in 96 deep well plates as described before (Mühle et al., 2012) all expression plasmids were transformed into the SCS-1 bacterial host (Agilent Technologies, USA) carrying the pS111 plasmid supplying rare arginine tRNAs and the lacI^q repressor (Brinkmann et al., 1989). Bacterial overnight cultures were used to inoculate TB medium and grown at 37 °C up to an OD_{600} of 1.0. Upon induction with 1 mM IPTG, cells were harvested after additional 3 h of growth. Purification of recombinant proteins followed a protocol recently described for the TM protein of FFV using sarcosyl extraction (PBS, 1% sarcosyl, 10 mM imidazol, pH 7.5) and refolding by dilution in PBS, 0.1% sarcosyl, 10 mM imidazol, pH 7.5 followed by subsequent NiNTA-affinity chromatography (Mühle et al., 2012).

2.3. SDS–PAGE and Western blot

Lysates of transfected 293T cells or transduced CRFK cells were prepared by lysis in M-PER extraction buffer (Thermo Scientific, Germany). 20 μg of total protein was separated by SDS–PAGE using the system described by Schägger and von Jagow, 1987 and then transferred to PVDF membranes (Millipore, USA) by semi-dry blotting. After blocking in 5% milk dissolved in PBS, 0.05% Tween 20 (PBS-T) for 1 h, membranes were probed either with 2F5 (Polymun Scientific, Austria, 0.3 μg/ml), a Bet specific antiserum (Mühle et al., 2013, 1:2000) or anti beta-actin (1:5000, Sigma–Aldrich, Germany) as primary antibodies followed by corresponding HRP-coupled secondary antibodies (Dako, Germany 1:3000). Blots were developed with ECL substrate and a digital imager (Intas, Germany).

2.4. Elisa

ELISA plates (Greiner BioOne, Austria) were coated with 200 ng of individual purified recombinant proteins diluted in water and incubated overnight at 37 °C. Dried plates were blocked with 5% BSA in PBS-T, washed once and then probed with 2F5 (0.3 μg/ml) or 4E10 (1.2 μg/ml) for 1 h at 37 °C before washing and incubation with an anti-human IgG-HRP conjugate (Dako, Germany, 1:3000). After six washing steps with PBS-T, plates were developed using OPD substrate (Sigma–Aldrich, Germany), stopped with 5 N sulphuric acid and read at 492 nm in a microtiter plate reader (Thermo Scientific, Germany).

2.5. Dot blot analysis

To assess binding of 2F5 and 4E10 under non-denaturing conditions, purified proteins were serially diluted in PBS with 0.1% sarcosyl and transferred to 0.45 μm PVDF membranes (Millipore, USA) by a vacuum manifold (Whatman, USA). In parallel, a synthetic HIV-1 FPPR-derived peptide (AAGSTMGAASMTLTVQARLLLSKKKK) and a MPER-derived peptide (KKKKEQELLELDKWASLWNWFNITNWL) both synthesised by GeneCust, Luxembourg were spotted as controls at equimolar concentrations. Membranes were then blocked, probed with 2F5 (0.3 μg/ml) or 4E10 (1.2 μg/ml), washed and developed as described above.

2.6. Construction of chimeric foamy viruses

A detailed description of the cloning process is given on Supplementary Fig. 2.

2.7. Virus production, passaging and titration

Infectious particles were produced by initial transfection of 293T cells with plasmid DNA or a GFP expressing vector (mock control) using a 1:3 w/w ratio of DNA and linear polyethylenimine. 48 h later, supernatants were collected, cleared by centrifugation and passed through 0.45 μm filters. After transfer to CRFK cells plated one day in advance, supernatants were removed after 4 h and fresh medium was added. In intervals of 3–4 days, medium was recovered, cleared and transferred to new cells as described above. Viruses of each passage were titrated by 5-fold serial dilution on FeFab indicator cells (Zemba et al., 2000) and subsequent staining with X-gal solution 72 h later. An ELISpot reader counted the number of infected cells visible as blue coloured spots as described recently (Mühle et al., 2011).

2.8. Virus sequencing

Proviruses were sequenced by extraction of genomic DNA from infected cells with the Blood and Tissue DNA Extraction Kit (Qiagen, Germany) followed by proof-reading PCR using primer I 5′-ataaccggtacacccaagacggatcctactcg-3′ located within Bet, primer LTR rev 5′-ggctctagatgtaagacggtgacttagctc-3′ and Phusion Hot Start Flex DNA Polymerase (NEB, USA). Amplicons were directly sequenced (Bet-His, Bet-E2) or subcloned (Bet-F2-M1, Bet-F2-M2) into the *Xmn*I blunted pMal-5pX vector for individual clone analysis.

3. Results

3.1. FPPR/MPER construct optimisation

In a first step, a set of recombinant antigens containing the MPER and the FPPR of the TM protein gp41 of HIV-1 connected by a linker was produced. Starting with a synthetic plasmid encoding the amino acid sequence of the construct F0-M0 and spacer amino acids (Supplementary Fig. 1), plasmids F0-M1 to F2-M2 having single amino acid deletions on the C- or N-terminus of the alpha-helical flanking regions were obtained by PCR-based mutagenesis (Fig. 1A , Supplementary Table 1). The effect of these deletions was analysed by alpha-helical wheel projections using the corresponding FPPR and MPER domains and flanking regions but excluding the loop residues (GTDS) which were identical in each construct. Assuming that both MPER and FPPR form helical structures as reported for the post-fusion/six-helix-bundle conformation (Buzon et al., 2010), deletions would result in a shifting of both domains against each other and potentially change epitope presentation (Fig 1B). The mutated plasmids were subcloned into a prokaryotic expression vector and recombinant N-terminally His-tagged proteins (Fig 1C) with a theoretical size of about 12.5 kDa were produced with typical yields of 4–5 mg/L. Characterisation of the purified proteins by SDS–PAGE and Coomassie blue staining demonstrated that the antigens had their expected size as well as a high degree of purity (Fig 2A). Initially, antigenicity of these proteins for 2F5 and 4E10 was examined by conventional ELISA. Slightly increased binding of 2F5 to antigens with deletions in the F-moiety was observed whereas enhanced affinity for 4E10 was evident for all M1 antigens and the F1-M2 protein (Fig. 2B). The differences found here were however weak and we hypothesised that antigen denaturation during coating might have affected binding characteristics. Reactivity of 2F5 and 4E10 with all antigens was therefore also analysed after serial dilution and non-denaturing transfer to membranes by vacuum. Here, notable

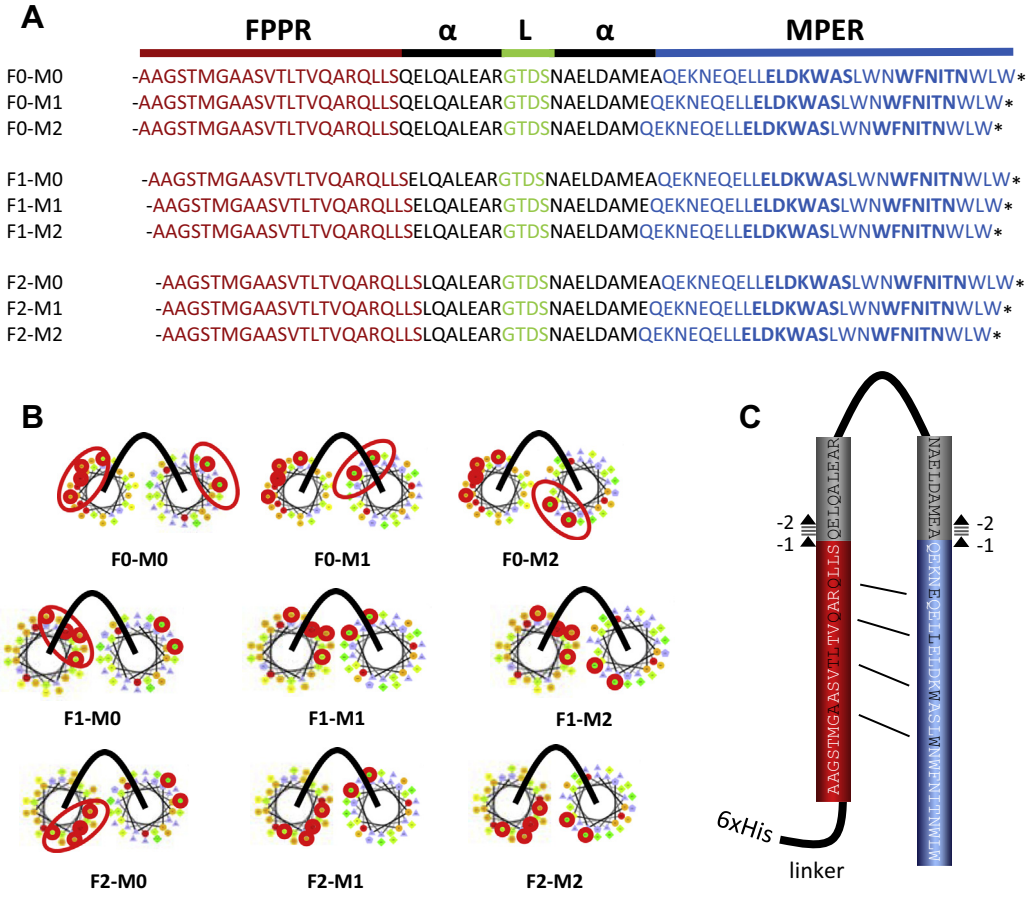

Fig. 1. Recombinant antigen sequences and design. (A) Amino acid sequences of the nine recombinant helix-loop-helix antigens comprising the fusion peptide proximal region (FPPR), the membrane proximal external region (MPER) connected by a loop element (L) and alpha-helical flanking amino acids (α). The epitopes of the broadly neutralising antibodies 2F5 (ELDKWAS) and 4E10 (WFNITN) in the MPER are highlighted in bold. Stop-codons are indicated by asterisks. The antigens are named corresponding to the number of amino acid deletions introduced into the flanking regions of the FPPR (F0, F1, F2) or MPER (M0, M1, M2). (B) Alpha-helical wheel projections of all antigens. Critical residues for 2F5 binding in the MPER (D and W residues of ELDKWA) and MPER contacting residues in the FPPR are marked as red circles to visualise the influence linker deletions on the positioning of these selected amino acids. Projections were made with the help of the Wheel program provided by Don Armstrong (http://trimer.tamu.edu/cgibin/wheel/wheel.pl) using separate predictions for FPPR and MPER domains with their respective flanking regions. The linking loop region identical in all antigens is indicated in black. (C) Schematic presentation of the loop antigen F0-M0. For purification, an N-terminal His-tag (His) was introduced after a Gly-Ala rich sequence (linker). The position of the amino acid shifting/deletion is indicated by arrows and numbers (−1/−2).

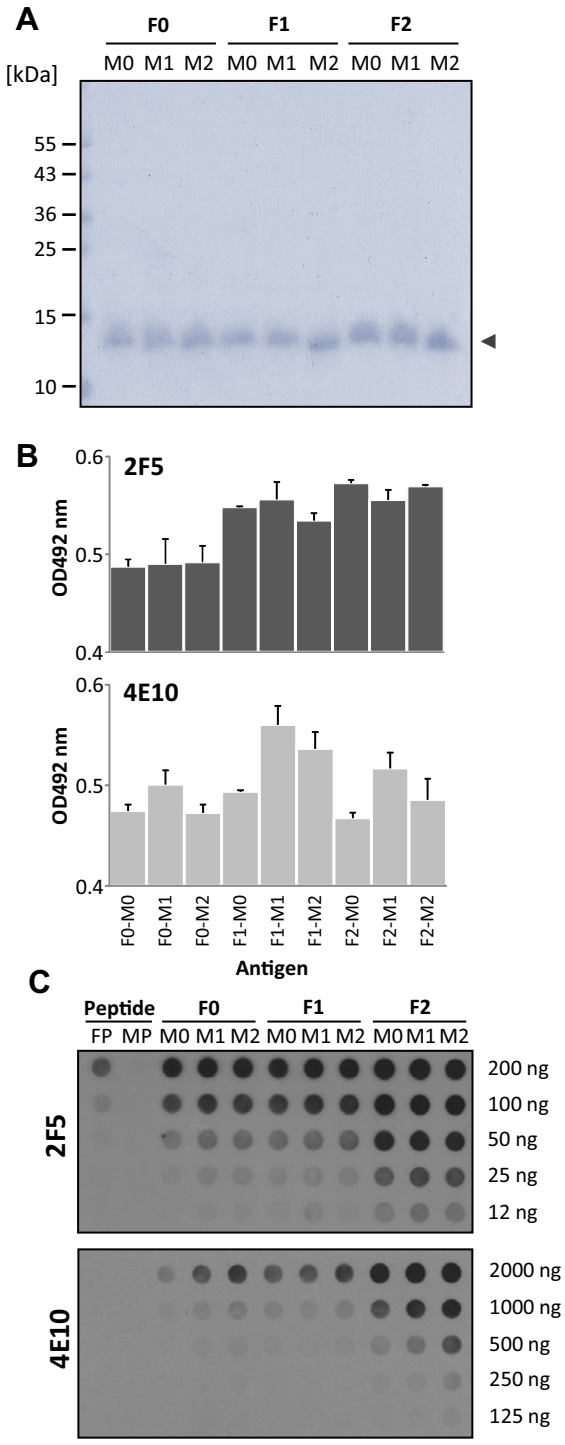

Fig. 2. Antigen purification and characterisation. (A) Purity of recombinant proteins after Ni–NTA affinity purification assessed by SDS–PAGE analysis and Coomassie blue staining. The arrow indicates the proteins at the expected molecular weight of about 12.5 kDa. (B) ELISA reactivity of indicated recombinant antigens with 2F5 (0.3 μg/ml) and 4E10 (1.2 μg/ml). (C) Binding of the monoclonal antibody 2F5 (0.3 μg/ml) and 4E10 (1.2 μg/ml) to serially diluted FPPR-MPER proteins or FPPR- and MPER-derived peptides (FP, MP), each transferred under non-denaturing conditions to PVDF membranes by vacuum at indicated protein concentrations. Deletions in the FPPR flanking region permit a clearly enhanced binding of 2F5 and to a lesser extend also for 4E10 whereas modification of MPER flanking residues has minor impact.

differences in immune recognition were observed (Fig. 2C). Deletions in the linker near the FPPR resulted in increased 2F5 binding, whereas modification of the linker near the MPER had only a minor influence. Antigens containing two deletions in the FPPR flanking regions (F2-X antigens) were superior to antigens having no or single deletions as they gave signals down to concentrations of 12 ng, whereas detection of other antigens by the 2F5 bnAb started to fade already at 50 ng (Fig 2C). A similar trend was observed in case of 4E10, although overall reactivity with the antigens was weak, requiring about tenfold more protein and lower antibody dilutions (Fig. 2C and Material and methods). Interestingly, although equimolar amounts were spotted, the FPPR-MPER antigens were better recognised compared to a synthetic MPER peptide alone. Conversely, a FPPR-derived peptide did not react as expected. These data indicated that the epitopes of 2F5 and 4E10 in the MPER have a favorable conformation when the FPPR-linker was shortened by two amino acids.

3.2. Construction and analysis of chimeric FFV

Based on these results, constructs F2-M0 to F2-M2 were selected for subcloning into the FFV infectious clone pCF-7 (Schwantes et al., 2002). In parallel the MPER alone was introduced (Fig 3A). Chimeric viruses were constructed by two sequential cloning steps (i) introducing Asp and Ser residues encoding a *Nhe*I restriction site and a C-terminal His-tag directly before the Bet stop codon (Bet-His) and (ii) introducing HIV-1 MPER or FPPR-MPER sequences and spacer amino acids into the Bet-His backbone by replacing the His-tag sequence (Fig 3A, see Supplementary Fig 2 for cloning details). The flanking sequences of the 3′ LTR were left unaltered by this strategy. Transfection of these chimeric vectors into 293T cells and harvest of supernatants 48 h later gave rise to progeny virus able to infect feline FeFab indicator cells at titres comparable to that of the minimally modified Bet-His construct (Fig 3B and C). Importantly, transfected 293T as well as the transduced CRFK cells produced the intended fusion proteins with their correct sizes of 46 (Bet), 51 (Bet-E2) and 56 kDa (Bet-FPPR-MPER antigens) as judged by immunoblotting with 2F5 or a Bet specific antiserum (Fig 3D). Notably, the enhanced recognition observed with recombinant fusion proteins was compensated by a reduced expression of the slightly larger Bet-FPPR-MPER proteins when compared to the Bet-E2 antigen (Fig 3D). Since stable production of the transgene is a prerequisite for studying processes of affinity maturation, we examined if expression is maintained during extended passaging on feline CRFK cells. Cleared supernatants from infected cells were transferred for ten passages (35 days in total) to uninfected CRFK cells and titrated in parallel on FeFab indicator cells. The titres of the construct Bet-His steadily increased upon serial passages and finally stabilised at 10^6 IU/ml (Fig. 4). In contrast, chimeric viruses carrying the HIV-1 epitopes behaved differently dependent on the cloned insert. An initial decrease of viral titres was observed until the 3rd and 4th passage, which was modest in chimeras Bet-E2 and Bet-F2-M1 (2×10^3 IU/ml) and more pronounced for constructs Bet-F2-M0 and Bet-F2-M2 (5×10^2 IU/ml). However, starting with passage 3 and 5 all titres except for the Bet-F2-M0 chimera continuously recovered to levels comparable to the Bet-His hybrid and also reached 1×10^6 IU/ml in passage ten (Fig 4A). This pattern indicated that adaptions within FFV were probably necessary to compensate insert expression, which are characterised by high hydrophobicity. To assure that these alterations did not compromise the HIV-1 epitope, cells from the last passage were again analysed by immunoblot using 2F5 (Fig 4B). Compared to passage one (Fig 3D) no differences in fusion protein size or recognition were observed for the Bet-E2, Bet-F2-M1 and Bet-F2-M2 constructs, whereas expression of the hybrid Bet-F2-M0 protein vanished after three passages as judged by a Bet-specific antiserum (Fig 4B and data not shown). We assume that the HIV-1 fusion part in this construct may be misfolded, impairing the ability of Bet to counteract APOBEC restriction in CRFK cells

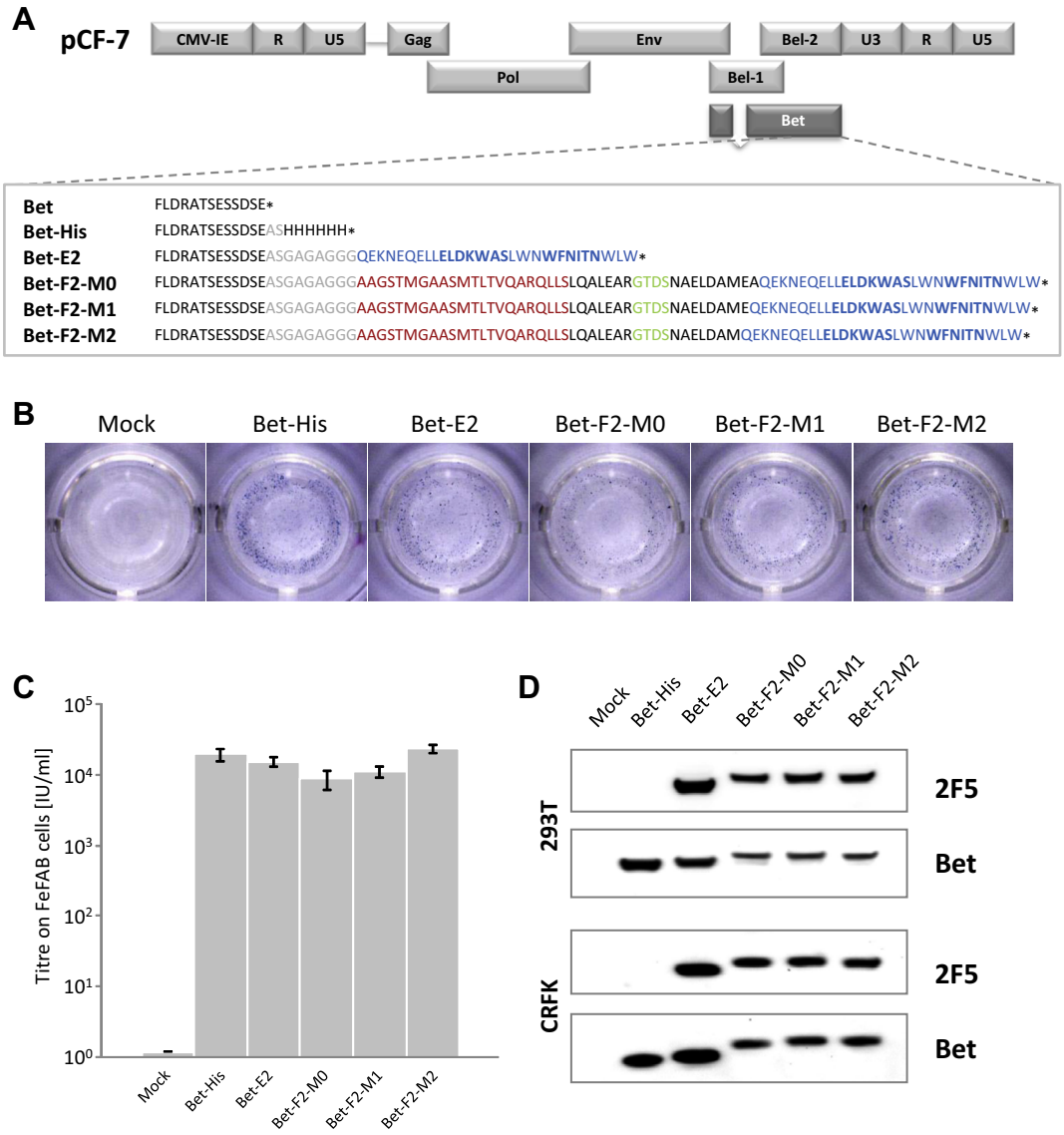

Fig. 3. Construction and characterisation of chimeric FFV. (A) Schematic illustration of the FFV molecular clone pCF-7 and amino acids introduced in frame to the C-terminus of Bet. The flanking 3′ LTR sequences were left unaltered. Between Bet and inserts, spacer amino acids (AS for Bet-His, ASGAGAGGG for all other constructs) were cloned. Labelling of the HIV-1 MPER (E2) or FPPR and MPER domains is corresponding to that in Figure. 1. Stop-codons are indicated by asterisks. (B) Infectivity of chimeric viruses or a GFP expressing vector (mock control) on feline cells. Cleared supernatants from transfected 293T were transferred to FeFab indicator cells which were stained 3 days later with X-gal solution to verify infection. (C) Titration of supernatants from transfected 293T cells on FeFab indicator cells. Progeny viruses with titres comparable to that of pCF-7 containing only a His-tag were obtained. (D) Transgene expression in transfected 293T and directly transduced CRFK cells using supernatants from transfected 293T cells. Cell lysates were prepared two days post transfection or three days after transduction, separated by SDS–PAGE, immunoblotted and then probed with 2F5 or a Bet specific antiserum. Transgene expression was specifically detected with 2F5 in all chimeric constructs containing the MPER but not the Bet-His or GFP expressing vectors (mock control).

which results in reduced viral replication (Chareza et al., 2012; Münk et al., 2010; Slavkovic Lukic et al., 2013). However, epitope presentation and size of the molecule in the other constructs remained stable for more than one month in culture, suggesting that these FFV/HIV-1 chimeras are most suitable for long-term antigen delivery.

4. Discussion

Convincing evidence has accumulated, showing that most HIV-1 bnAb are unique in terms of antibody genetics, structure, epitope recognition and their mode of emergence. As they are not easily elicited by classical immunisation regimens, different factors including affinity maturation seem to influence their induction. We here aimed to develop a vector system based on replicating FV to allow extended HIV-1 antigen delivery and designed a set of HIV-1 MPER antigens linked to potentially constraining FPPR residues. By systematic shortening of linker residues, some of the antigens showed enhanced recognition by 2F5 and 4E10, indicating that a certain positioning of both domains is essential for improved binding (Fig. 2). Importantly, the affinity of 2F5 to its epitope could be markedly improved, whereas the binding of 4E10 remained weak as observed before (Mühle et al., 2013). Interestingly, there were differences in binding dependent on whether the antigens were denatured once (ELISA) or were kept in solution (dot blot assay). This implies that conformation sensitive structures are formed when the antigens are in a native-like environment. Notably, antigenicity did not correlate well with epitope accessibility predicted by helical

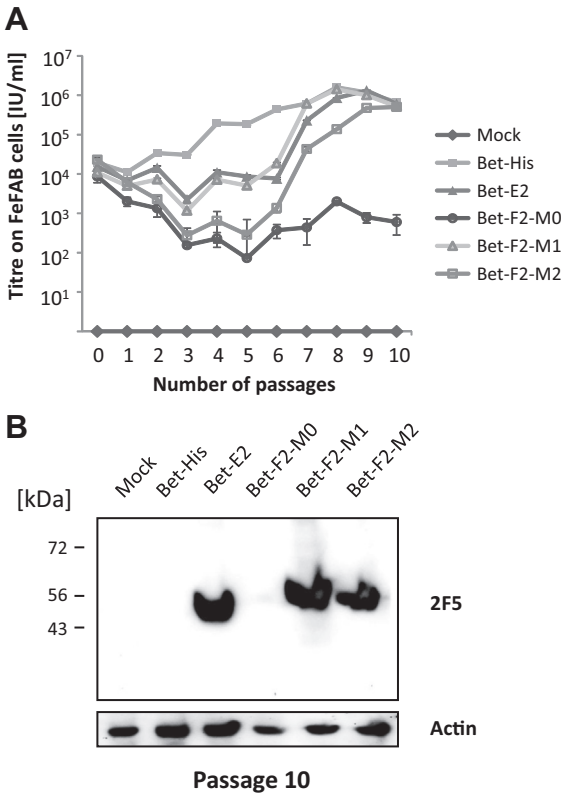

Fig. 4. Viral titres and transgene expression after extended passaging on feline CRFK cells. (A) Titres of chimeric viruses or a GFP expressing vector (mock control) titrated on FeFab indicator cells after the number of indicated passages. (B) Transgene expression after passage 10 as detected using the 2F5 antibody. Protein loading was verified using an antibody against beta-actin.

wheel projections, which assume an ideal alpha-helical character of the FPPR and MPER and consider that both domains are independent. Interhelical interactions or formation of non-helical/beta-turn structures in MPER caused by the presence of FPPR residues (de la Arada et al., 2009) may reduce the degree of epitope shifting in the actual structure here. When the inserts were introduced into the infectious FFV clone, the chimeras were replication-competent with nearly identical titres and infectivity. However, upon passaging on CRFK cells an initial decline of viral titres was detected before stabilisation at the same level as the Bet-His control in three of the four constructs. In previous studies using Bet fusion proteins, genetic instability of chimeric vectors and expression of truncated proteins has been observed, which was inversely correlated with insert size (Schwantes et al., 2002, 2003). The pattern seen here also suggested adaptations, however, HIV-1 epitope expression was obviously not affected (Fig 4B). When proviral sequences from passage 10 from all constructs except the Bet-F2-M0 vector from which no amplicon could be obtained were sequenced, no and up to four independent amino acid changes within Bet were observed in case of Bet-His and the Bet-E2 vectors, respectively, but the HIV epitopes were unchanged (data not shown). The other two Bet-F2-MX vectors were sequenced after cloning. Seven (Bet-F2-M1) and nine (Bet-F2-M2) of 11 clones analysed encoded the entire HIV MPER epitope, whereas the remaining clones expressed truncated forms of Bet due to premature stop codons or insert deletion and fusion to LTR sequences (data not shown). As a next step, it will be essential to investigate the behaviour of these vectors *in vivo* to test their replicative potential and capability to achieve affinity maturation.

5. Conclusions

Replication-competent FV constructs stably expressing the epitopes recognised by antibodies broadly neutralising HIV-1 were developed. These constructs will allow a first evaluation of their immunogenicity in cats and, if successful, justify the transfer of the strategy to the Bet protein of primate FV to test applicability in primates.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.antiviral.2013.09.009.

References

Alke, A., Schwantes, A., Zemba, M., Flügel, R.M., Löchelt, M., 2000. Characterization of the humoral immune response and virus replication in cats experimentally infected with feline foamy virus. Virology 275, 170–176.
Balazs, A.B., Chen, J., Hong, C.M., Rao, D.S., Yang, L., Baltimore, D., 2012. Antibody-based protection against HIV infection by vectored immunoprophylaxis. Nature 481, 81–84.
Bleiholder, A., Mühle, M., Hechler, T., Bevins, S., Vandewoude, S., Denner, J., Löchelt, M., 2011. Pattern of seroreactivity against feline foamy virus proteins in domestic cats from Germany. Vet Immunol Immunopathol. 143, 292–300.
Brinkmann, U., Mattes, R.E., Buckel, P., 1989. High-level expression of recombinant genes in Escherichia coli is dependent on the availability of the dnaY gene product. Gene 85, 109–114.
Bryson, S., Julien, J.P., Hynes, R.C., Pai, E.F., 2009. Crystallographic definition of the epitope promiscuity of the broadly neutralizing anti-human immunodeficiency virus type 1 antibody 2F5: vaccine design implications. J. Virol. 83, 11862–11875.
Burton, D.R., Stanfield, R.L., Wilson, I.A., 2005. Antibody vs. HIV in a clash of evolutionary titans. Proc. Natl. Acad. Sci. USA 102, 14943–14948.
Buzon, V., Natrajan, G., Schibli, D., Campelo, F., Kozlov, M.M., Weissenhorn, W., 2010. Crystal structure of HIV-1 gp41 including both fusion peptide and membrane proximal external regions. PLoS Pathog. 6, e1000880.
Callahan, M.E., Switzer, W.M., Matthews, A.L., Roberts, B.D., Heneine, W., Folks, T.M., Sandstrom, P.A., 1999. Persistent zoonotic infection of a human with simian foamy virus in the absence of an intact orf-2 accessory gene. J. Virol. 73, 9619–9624.
Chareza, S., Slavkovic Lukic, D., Liu, Y., Rathe, A.M., Munk, C., Zabogli, E., Pistello, M., Lochelt, M., 2012. Molecular and functional interactions of cat APOBEC3 and feline foamy and immunodeficiency virus proteins: different ways to counteract host-encoded restriction. Virology 424, 138–146.
de la Arada, I., Julien, J.P., de la Torre, B.G., Huarte, N., Andreu, D., Pai, E.F., Arrondo, J.L., Nieva, J.L., 2009. Structural constraints imposed by the conserved fusion peptide on the HIV-1 gp41 epitope recognized by the broadly neutralizing antibody 2F5. J. Phys. Chem. B 113, 13626–13637.
Deyle, D.R., Khan, I.F., Ren, G., Russell, D.W., 2013. Lack of genotoxicity due to foamy virus vector integration in human iPSCs. Gene Ther. 20, 868–873.
Doria-Rose, N.A., Klein, R.M., Manion, M.M., O'Dell, S., Phogat, A., Chakrabarti, B., Hallahan, C.W., Migueles, S.A., Wrammert, J., Ahmed, R., Nason, M., Wyatt, R.T., Mascola, J.R., Connors, M., 2009. Frequency and phenotype of human immunodeficiency virus envelope-specific B cells from patients with broadly cross-neutralizing antibodies. J. Virol. 83, 188–199.
Ferrantelli, F., Rasmussen, R.A., Buckley, K.A., Li, P.L., Wang, T., Montefiori, D.C., Katinger, H., Stiegler, G., Anderson, D.C., McClure, H.M., Ruprecht, R.M., 2004. Complete protection of neonatal rhesus macaques against oral exposure to pathogenic simian-human immunodeficiency virus by human anti-HIV monoclonal antibodies. J. Infect. Dis. 189, 2167–2173.
Fiebig, U., Schmolke, M., Eschricht, M., Kurth, R., Denner, J., 2009. Mode of interaction between the HIV-1-neutralizing monoclonal antibody 2F5 and its epitope. AIDS 23, 887–895.
Giron, M.L., de The, H., Saib, A., 1998. An evolutionarily conserved splice generates a secreted env-Bet fusion protein during human foamy virus infection. J. Virol. 72, 4906–4910.
Gray, E.S., Madiga, M.C., Hermanus, T., Moore, P.L., Wibmer, C.K., Tumba, N.L., Werner, L., Mlisana, K., Sibeko, S., Williamson, C., Abdool Karim, S.S., Morris, L., Team, C.S., 2011. The neutralization breadth of HIV-1 develops incrementally over four years and is associated with CD4+ T cell decline and high viral load during acute infection. J. Virol. 85, 4828–4840.

Haynes, B.F., Kelsoe, G., Harrison, S.C., Kepler, T.B., 2012. B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. Nat. Biotechnol. 30, 423–433.
Heneine, W., Schweizer, M., Sandstrom, P., Folks, T., 2003. Human infection with foamy viruses. Curr. Top. Microbiol. Immunol. 277, 181–196.
Heneine, W., Switzer, W.M., Sandstrom, P., Brown, J., Vedapuri, S., Schable, C.A., Khan, A.S., Lerche, N.W., Schweizer, M., Neumann-Haefelin, D., Chapman, L.E., Folks, T.M., 1998. Identification of a human population infected with simian foamy viruses. Nat. Med. 4, 403–407.
Hessell, A.J., Rakasz, E.G., Poignard, P., Hangartner, L., Landucci, G., Forthal, D.N., Koff, W.C., Watkins, D.I., Burton, D.R., 2009. Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. PLoS Pathog. 5, e1000433.
Hessell, A.J., Rakasz, E.G., Tehrani, D.M., Huber, M., Weisgrau, K.L., Landucci, G., Forthal, D.N., Koff, W.C., Poignard, P., Watkins, D.I., Burton, D.R., 2010. Broadly neutralizing monoclonal antibodies 2F5 and 4E10 directed against the human immunodeficiency virus type 1 gp41 membrane-proximal external region protect against mucosal challenge by simian-human immunodeficiency virus SHIVBa-L. J. Virol. 84, 1302–1313.
Huang, J., Ofek, G., Laub, L., Louder, M.K., Doria-Rose, N.A., Longo, N.S., Imamichi, H., Bailer, R.T., Chakrabarti, B., Sharma, S.K., Alam, S.M., Wang, T., Yang, Y., Zhang, B., Migueles, S.A., Wyatt, R., Haynes, B.F., Kwong, P.D., Mascola, J.R., Connors, M., 2012. Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. Nature 491, 406–412.
Julien, J.P., Bryson, S., Nieva, J.L., Pai, E.F., 2008. Structural details of HIV-1 recognition by the broadly neutralizing monoclonal antibody 2F5: epitope conformation, antigen-recognition loop mobility, and anion-binding site. J. Mol. Biol. 384, 377–392.
Lecellier, C.H., Vermeulen, W., Bachelerie, F., Giron, M.L., Saib, A., 2002. Intra- and intercellular trafficking of the foamy virus auxiliary bet protein. J. Virol. 76, 3388–3394.
Lindemann, D., Rethwilm, A., 1998. Characterization of a human foamy virus 170-kilodalton Env-Bet fusion protein generated by alternative splicing. J. Virol. 72, 4088–4094.
Lindemann, D., Rethwilm, A., 2011. Foamy virus biology and its application for vector development. Viruses 3, 561–585.
Liu, W., Lei, J., Liu, Y., Lukic, D.S., Rathe, A.M., Bao, Q., Kehl, T., Bleiholder, A., Hechler, T., Lochelt, M., 2013. Feline foamy virus-based vectors: advantages of an authentic animal model. Viruses 5, 1702–1718.
Lutje Hulsik, D., Liu, Y.Y., Strokappe, N.M., Battella, S., El Khattabi, M., McCoy, L.E., Sabin, C., Hinz, A., Hock, M., Macheboeuf, P., Bonvin, A.M., Langedijk, J.P., Davis, D., Forsman Quigley, A., Aasa-Chapman, M.M., Seaman, M.S., Ramos, A., Poignard, P., Favier, A., Simorre, J.P., Weiss, R.A., Verrips, C.T., Weissenhorn, W., Rutten, L., 2013. A gp41 MPER-specific llama VHH requires a hydrophobic CDR3 for neutralization but not for antigen recognition. PLoS Pathog. 9, e1003202.
Mascola, J.R., Montefiori, D.C., 2010. The role of antibodies in HIV vaccines. Annu. Rev. Immunol. 28, 413–444.
Montero, M., van Houten, N.E., Wang, X., Scott, J.K., 2008. The membrane-proximal external region of the human immunodeficiency virus type 1 envelope: dominant site of antibody neutralization and target for vaccine design. Microbiol. Mol. Biol. Rev. 72, 54–84, table of contents.
Mühle, M., Bleiholder, A., Kolb, S., Hübner, J., Löchelt, M., Denner, J., 2011. Immunological properties of the transmembrane envelope protein of the feline foamy virus and its use for serological screening. Virology 412, 333–340.
Mühle, M., Hoffmann, K., Löchelt, M., Denner, J., 2013. Immunisation with foamy virus Bet fusion proteins as novel strategy for HIV-1 epitope delivery. Immunol. Res. 56, 61–72.
Mühle, M., Löchelt, M., Denner, J., 2012. Optimisation of expression and purification of the feline and primate foamy virus transmembrane envelope proteins using a 96 deep well screen. Protein Expr. Purif. 81, 96–105.
Münk, C., Hechler, T., Chareza, S., Löchelt, M., 2010. Restriction of feline retroviruses: lessons from cat APOBEC3 cytidine deaminases and TRIM5alpha proteins. Vet. Immunol. Immunopathol. 134, 14–24.
Muster, T., Steindl, F., Purtscher, M., Trkola, A., Klima, A., Himmler, G., Ruker, F., Katinger, H., 1993. A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1. J. Virol. 67, 6642–6647.
Noah, E., Biron, Z., Naider, F., Arshava, B., Anglister, J., 2008. The membrane proximal external region of the HIV-1 envelope glycoprotein gp41 contributes to the stabilization of the six-helix bundle formed with a matching N' peptide. Biochemistry 47, 6782–6792.
Ofek, G., Tang, M., Sambor, A., Katinger, H., Mascola, J.R., Wyatt, R., Kwong, P.D., 2004. Structure and mechanistic analysis of the anti-human immunodeficiency virus type 1 antibody 2F5 in complex with its gp41 epitope. J. Virol. 78, 10724–10737.
Romen, F., Pawlita, M., Sehr, P., Bachmann, S., Schroder, J., Lutz, H., Lochelt, M., 2006. Antibodies against Gag are diagnostic markers for feline foamy virus infections while Env and Bet reactivity is undetectable in a substantial fraction of infected cats. Virology 345, 502–508.
Sather, D.N., Armann, J., Ching, L.K., Mavrantoni, A., Sellhorn, G., Caldwell, Z., Yu, X., Wood, B., Self, S., Kalams, S., Stamatatos, L., 2009. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. J. Virol. 83, 757–769.
Schägger, H., von Jagow, G., 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal. Biochem. 166, 368–379.
Schwantes, A., Ortlepp, I., Lochelt, M., 2002. Construction and functional characterization of feline foamy virus-based retroviral vectors. Virology 301, 53–63.
Schwantes, A., Truyen, U., Weikel, J., Weiss, C., Lochelt, M., 2003. Application of chimeric feline foamy virus-based retroviral vectors for the induction of antiviral immunity in cats. J. Virol. 77, 7830–7842.
Slavkovic Lukic, D., Hotz-Wagenblatt, A., Lei, J., Rathe, A.M., Muhle, M., Denner, J., Munk, C., Lochelt, M., 2013. Identification of the feline foamy virus Bet domain essential for APOBEC3 counteraction. Retrovirology 10, 76.
Stiegler, G., Kunert, R., Purtscher, M., Wolbank, S., Voglauer, R., Steindl, F., Katinger, H., 2001. A potent cross-clade neutralizing human monoclonal antibody against a novel epitope on gp41 of human immunodeficiency virus type 1. AIDS Res. Hum. Retroviruses 17, 1757–1765.
Trobridge, G.D., 2009. Foamy virus vectors for gene transfer. Expert Opin. Biol. Ther. 9, 1427–1436.
Trobridge, G.D., Miller, D.G., Jacobs, M.A., Allen, J.M., Kiem, H.P., Kaul, R., Russell, D.W., 2006. Foamy virus vector integration sites in normal human cells. Proc. Natl. Acad. Sci. USA 103, 1498–1503.
Zemba, M., Alke, A., Bodem, J., Winkler, I.G., Flower, R.L., Pfrepper, K., Delius, H., Flugel, R.M., Lochelt, M., 2000. Construction of infectious feline foamy virus genomes: cat antisera do not cross-neutralize feline foamy virus chimera with serotype-specific Env sequences. Virology 266, 150–156.
Zhou, M., Meyer, T., Koch, S., Koch, J., von Briesen, H., Benito, J.M., Soriano, V., Haberl, A., Bickel, M., Dubel, S., Hust, M., Dietrich, U., 2013. Identification of a new epitope for HIV-neutralizing antibodies in the gp41 membrane proximal external region by an Env-tailored phage display library. Eur. J. Immunol. 43, 499–509.