AvFc, a novel Fc fusion protein targeting Env high-mannose glycans

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OBJECTIVE

Investigate the feasibility of **Avaren-Fc (AvFc), a high-mannose glycan-specific human antibody-like protein,** for use in HIV prevention and/or treatment.

INTRODUCTION

Background

- Growing evidence suggests that high-mannose glycans on HIV envelope constitute a promising target for anti-HIV strategies.[1-3]
- Induction of high-mannose glycan-binding antibodies through vaccination is challenging due to the poor immunogenicity and low antigenicity of carbohydrate epitopes.
- Actinohivin (AH) is an actinomycete-derived lectin specific to high-mannose glycans and shows nanomolar anti-HIV activity without mitogenicity or cytotoxicity in human PBMC.[4]
- AH may therefore provide a novel platform for anti-viral therapeutics.

AvFc

- A fusion protein consisting of Avaren and a human IgG1 Fc region (Fig. 1).
- Avaren is a highly bioproducible variant of AH (Fig. 3).
- Exhibits high specificity to oligomannose glycans containing α1,2-linked mannobiose (Fig. 4).
- Has low-nanomolar anti-HIV activity against multiple HIV strains (Fig. 5).
- Efficiently manufactured in *Nicotiana benthamiana* tobacco plants (Fig. 4).

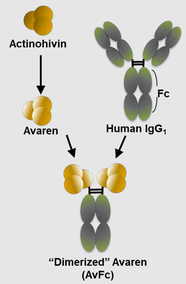


Figure 1. Schematic diagram showing AvFc engineering.

Protein production in plants[5]

- Compatible with complex protein assembly and post-translational modifications (e.g., monoclonal antibodies).
- Rapid high-level protein accumulation in leaf tissue using plant virus vectors (within days).
- Potential for economical large-scale manufacturing.
- Less likely contaminated with human pathogens.



Figure 2. *N. benthamiana* in an environment-controlled facility at Kentucky BioProcessing, Inc.

RESULTS

1. Molecular engineering and production of AvFc

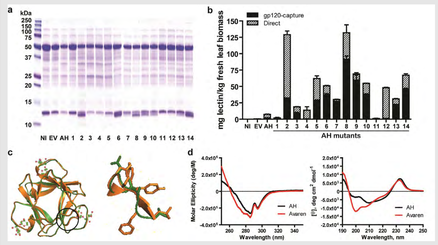
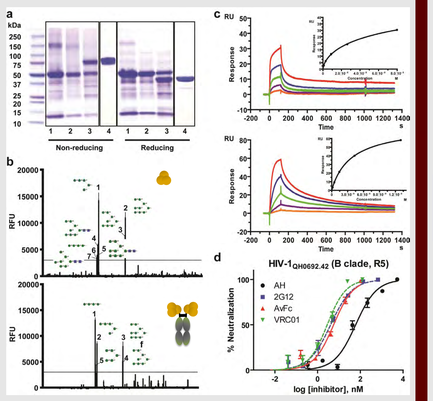


Figure 3. a. SDS-PAGE analysis of *N. benthamiana* leaves expressing AH mutants. **b**. Quantification of AH mutants in leaf tissue by direct or gp120-capture ELISA. **c**. AH's crystal structure (PDB ID: 4G1R) (orange) superimposed with a homology model of Avaren (green). **d.** Far-UV (left) and near-UV (right) circular dichroism spectra of AH and Avaren.

Figure 4. a. SDS-PAGE analysis of AvFc. Lane 1: Control leaf proteins. Lane 2: Empty vector infiltrated leaf proteins. Lane 3: AvFc expressing leaf proteins. Lane 4: 10 µg of purified AvFc. **b.** Glycan array analysis of AH (top) and AvFc (bottom) in a mammalian glycan array with 610 glycans. **c.** Surface plasmon resonance analysis of the binding of AH (top) and AvFc (bottom) to recombinant HIV-1 $gp120_{SF162}$. Equilibrium dissociation constants, K_D, were determined based on steady state (inset) to be 29.1 ± 0.055 nM (AH) and 3.54 ± 0.011 nM (AvFc). **d**. An Env-pseudotyped virus neutralization assay of AH, AvFc, 2G12 and VRC01.



2. HIV-neutralizing and Fc receptor -mediated activities of AvFc

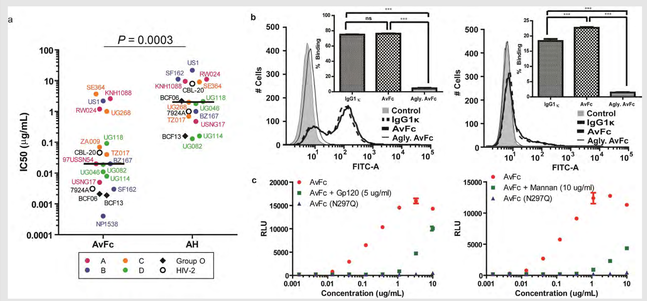


Figure 5. a. Human peripheral blood mononucleated cell (PBMC) based primary HIV neutralization assay. Infectivity was measure by p24 amounts. **b**. Flow cytometry analysis of AvFc's binding to human Fcγ receptors. TZM-bl cells expressing Human FcγRI (left) or FcγRIIIA (right) were incubated with either human IgG1κ isotype control, AvFc, or aglycosylated AvFc (Agly. AvFc), each at a final concentration of 1 µg/mL for FcγRI and 100 µg/mL for FcγRIIIA. **c.** ADCC activity of AvFc. A reporter-gene (luciferase) expression bioassay based on FcγIIIA+ Jurkat cells and Env+ HeLa cells was used to assess AvFc's ADCC activity. AvFc (N297Q): aglycosylated AvFc.

3. AvFc's cytotoxicity, mitogenicity and pro-inflammatory potential in human PBMC

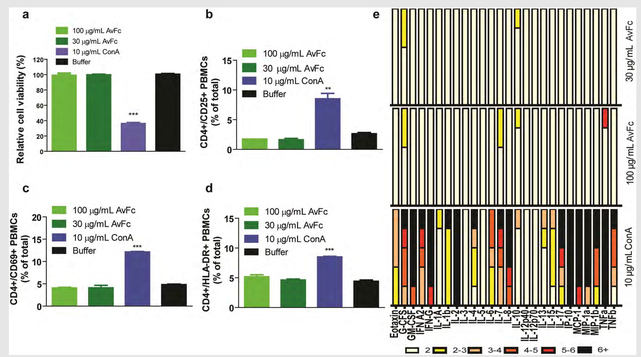


Figure 6. a. PBMC viability, assessed by flow cytometry after staining with propidium iodide. **b - d**. CD4+ cell activation, as analyzed by the surface expression of CD25 (b), CD69 (c) and HLA-DR (d). **e.** Cytokine and chemokine secretion assessed by a multiplex bead array.

4. AvFc's safety upon intravaginal and intravenous administration.

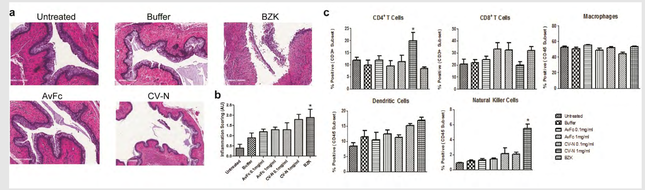
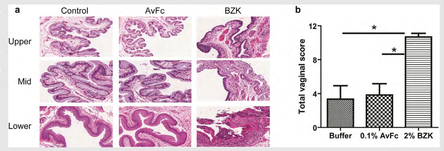


Figure 7. Mouse vaginal administration. Balb/c mice treated intravaginally with 1 mg/mL of AvFc, CV-N, 2% benzalkonium chloride (BZK) or vehicle control for 5 consecutive days. **a**. Representative hematoxylin & eosin (H&E)-stained mouse vaginal sections. **b**. Total irritation scores based on epithelial ulceration, edema, leukocyte influx, and vascular congestion in the vagina. **c**. Flow cytometry analysis of immune cell populations. ANOVA with Bonferroni post tests was performed; *P < 0.05, compared to the buffer control.

Figure 8. Rabbit 10-day vaginal irritation assay. a. Representative H&E-stained sections of the upper, mid, and lower vagina. **b**. Total irritation scores. Data are expressed as mean±SEM of 3 rabbits.



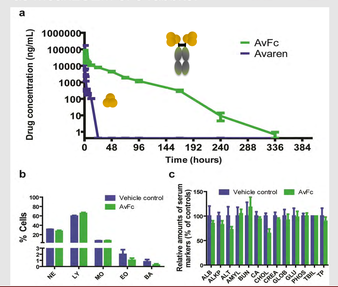


Figure 9. Rat single-dose (4 mg/kg) intravenous administration study. a. Avaren and AvFc in serum were detected by gp120-capture ELISA. **b**. Complete blood counts 14 days after administration. Data are expressed as mean±SEM of 3 rabbits.
NE: neutrophil; LY: lymphocyte; MO: monocyte; EO: eosinophil; and BA: basophil. Data represents mean ± SEM of four animals. **c**. Serum chemistry. ALB: albumin; ALKP: alkaline phosphatase; ALT: alanine-aminotransferase; AMYL: amylase; BUN: blood urea nitrogen; CA: calcium; CHOL: cholesterol; CREA: creatinine; GLOB: globulin; GLU: glucose: PHOS: phosphorus; TBIL: total bilirubin; and TP: total protein.

4. Anti-HIV synergism between AvFc and other antiviral agents

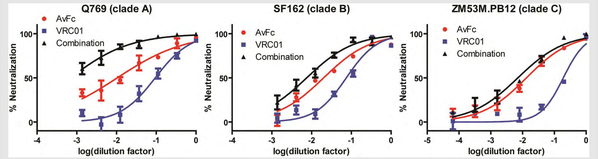


Figure 10. Analysis of synergism between AvFc and VRC01. The degree of synergism was analyzed by the CalcuSyn software (Biosoft) based on the multiple-drug effect equation. Mean combination index (CI) values at IC_{50} levels were 0.27 ± 0.09 (Q769), 0.58 ± 0.15 (SF162) and 0.24 ± 0.07 (ZM53M.PB12), respectively.

Table 1. Combination index (CI)* values for AvFc in combination with Tenofovir and Maraviroc

HIV strain	Mean CI ± SEM		
	IC_{50}	IC_{75}	IC_{90}
SF162 (clade B)			
Tenofovir	0.58 ± 0.09	0.49 ± 0.17	0.51 ± 0.26
Maraviroc	0.58 ± 0.22	0.43 ± 0.15	0.34 ± 0.07
CAP210.2.00.E8 (clade C)			
Tenofovir	0.79 ± 0.23	0.57 ± 0.08	0.61 ± 0.14
Maraviroc	0.54 ± 0.19	0.28 ± 0.03	0.20 ± 0.05

*A CI of <0.9 indicates synergy, 0.9 to 1.1 indicates addition, and >1.1 indicates antagonism.

CONCLUSION

1. AvFc is a novel, highly bioproducible anti-HIV protein specific to Env high-mannose glycans.
2. AvFc exhibits broad nanomolar HIV-neutralizing activity and can induce Fc-mediated functions.
3. AvFc appears to be safe for vaginal and systemic administration.
4. AvFc synergizes with VRC01, tenofovir and maraviroc.

ACKNOWLEDGMENTS

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