Using Nanobodies as an Intracellular Biological to Block HIV-1 Through Innate Antiviral Mechanisms

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ABSTRACT

Hypothesis. Recent studies have explored antibodies targeting the HIV envelope as alternative therapies. Most of antibodies are broadly neutralizing antibodies and block binding and entry. Here we investigated the potential of antibody mediated intracellular immunity against HIV-1 infection, utilizing the intracellular E3 ubiquitin ligase and antibody receptor TRIM21. In the cytosol, the TRIM21 protein interacts with an antibody-coated virus and targets the complex for proteasomal degradation. Our hypothesis is that intracellular recognition of HIV-1 by antibodies results in degradation of viral particles. **Results.** Our data show that our Fc-modified nanobodies recognize intact HIV-1 capsid and have a functional Fc domain. Notably, HIV-1 p24 capsid was neither detected intracellularly nor extracellularly after HIV-1 exposure in the p24 nanobody-Fc expressing cell-line, even though HIV-1 had integrated in the genome at levels comparable to control cells. These results demonstrate that the nanobodies are blocking production of new virus particles, rather than targeting HIV-1 during initial infection. In cells transfected with the purified nanobody a similar decrease of p24 was observed. These data strongly suggest that the Fc-modified p24 nanobody actively degrades p24 and thereby prevents new virus production. **Conclusion.** We have developed a novel p24 nanobody that efficiently blocks HIV-1 production. Intracellular production, as well as transfection of Fc-modified p24 nanobodies completely reduces p24 production levels in cells upon infection with HIV-1.

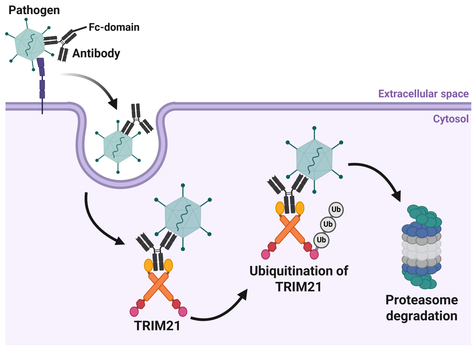


Fig. 1 TRIM21 mechanism. An antibody binds to a pathogen in the extracellular environment. If the pathogen manages to enter the cell, the attached antibody will be detected by the cytosolic Fc-receptor TRIM21. When this interaction results in the autoubitiquination of TRIM21, it leads to the proteasomal degradation of the whole complex.

p24-Fc nanobodies bind p24 capsid protein

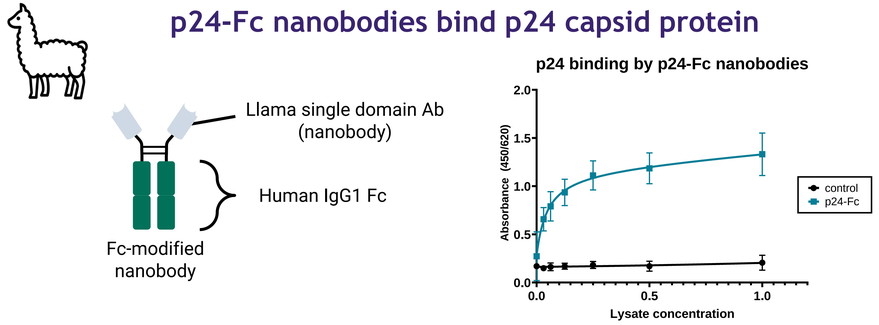


Fig. 2 (A) The single domain antibody (sdAb) from llama's is combined with human IgG1 Fc domain. **(B)** Lysates from U87.CD4.CCR5 cells that were transduced to express p24-Fc nanobodies were found to bind to intact HIV-1 capsid using a p24-capture ELISA, whereas cells transduced with a backbone control construct didn't show any binding.

p24-Fc nanobodies block HIV-1 production

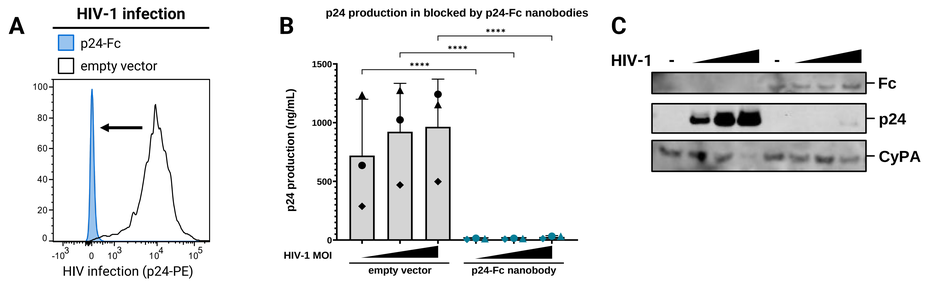
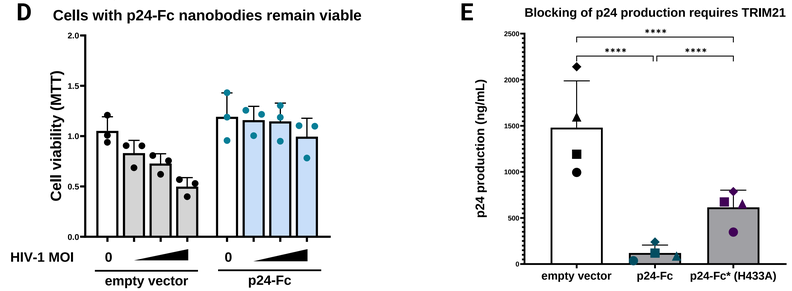


Fig. 3 (A) U87.CD4.CCR5 cells, transduced with a p24-Fc or control construct were infected with HIV-1 lab strain NL4.3-BaL and analyzed after 5 days on flow cytometry for the presence of p24. In control cells, almost all cells were p24-positive, whereas almost all cells that produce the Fc-modified nanobodies are negative for p24. **(B)** In the same nanobody-expressing U87.CD4.CCR5 cells, after 7 days of infection with NL4.3BaL at different multiplicity of infection (MOI), no p24 was measured in supernatant using ELISA. Additional testing with Western Blot **(C)** also showed an absence of p24 in cells that produce Fc-modified nanobodies, whereas control cells contain increasingly more p24 with higher MOI. Cyclophilin A (CyPA) is used as a control gene.
(D) Viability was measured using an MTT assay that measures cellular metabolic activity as an indicated for viability. With increasing MOI of infection with NL4.3BaL, viability for control cells decreased, whereas viability for cells containing nanobodies remained similar to no virus control. **(E)** An Fc mutant (H433A) that can't bind TRIM21was generated using site-directed mutagenesis. U87.CD4.CCR5 transduced with these constructs were infected with NL4.3BaL and the amount of p24 was measured by ELISA. In cells producing p24-Fc (H433A) mutant, p24 production was partially restored, indicating the role of TRIM21 in this process.



Turning p24-Fc nanobodies into intracellular anti-HIV biologicals

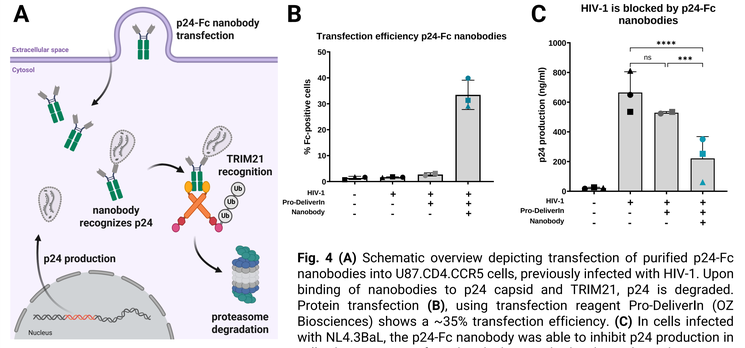


Fig. 4 (A) Schematic overview depicting transfection of purified p24-Fc nanobodies into U87.CD4.CCR5 cells, previously infected with HIV-1. Upon binding of nanobodies to p24 capsid and TRIM21, p24 is degraded. Protein transfection **(B)**, using transfection reagent Pro-DeliverIn (OZ Biosciences) shows a ~35% transfection efficiency. **(C)** In cells infected with NL4.3BaL, the p24-Fc nanobody was able to inhibit p24 production in cells that were transfected with the nanobody, shown by a decrease in p24 production in culture supernatant.

CONCLUSIONS

- We have developed a novel p24 nanobody that efficiently blocks HIV-1 infection
- Intracellular production yields functional Fc-modified p24 nanobodies that bind intact capsid
- The modified p24 nanobodies completely reduce intracellular p24 production levels in U87.CD4.CCR5 cells upon infection with HIV-1
- TRIM21 contributes to the decrease in p24 levels
- These p24 nanobodies can be used as biologicals
- Intracellular Fc-biologicals against Gag have the potential to be broadly neutralizing antibodies

This technology holds great promise for the engineering of therapeutic antibodies to prevent viral infections via targeting of host or viral proteins.

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