

POSTER 40**LLAMA SINGLE-DOMAIN ANTIBODY FRAGMENT FOR INHIBITION OF THE HIV-1 NEF PROTEIN**

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The Nef regulatory protein of HIV-1 is essential *in vivo* for virus replication and AIDS pathogenesis. Nef acts during the late stages of the viral life cycle to exert a positive influence on virus infectivity, and its role is related, at least in part, to its ability to induce profound morphological and functional perturbations of the endocytic pathway. These Nef-induced perturbations lead to dysregulation of endocytosis of many membrane proteins, including CD4, the main cellular receptor of HIV-1 in both T lymphocytes and macrophages. In order to develop specific tools and reagents able to inhibit the Nef action on endocytosis and virus infectivity in HIV-1 infected cells, we took advantages of the specific characteristics of the single-domain antibodies (sdAbs) from camelids. After immunization of a llama with a recombinant form of Nef, a library of sdAbs was constructed from the B-lymphocytes of the immunized-animal and then screened using the phage-display technique in *E. coli*. A specific anti-Nef sdAb with a high affinity ($K_d=1.5 \times 10^{-10}$ M) was thus isolated and selected for further biochemical and virological characterization. When expressed as an intracellular antibody ("intrabody") in HIV-1-infected cells, this anti-Nef sdAb inhibited most of the biological functions of Nef. First, it was able to counteract the CD4-downregulation activity of Nef through inhibition of the Nef effects on CD4 internalization from the cell surface. Second, anti-Nef sdAb did inhibit the requirement of Nef for the full infectivity of HIV-1. Experiments are in progress to analyze the inhibitory activity of the anti-Nef sdAb in HIV-1 transgenic mice expressing Nef, and preliminary results suggest that it is working in this animal model to revert the Nef-induced alterations of the immune functions. In conclusion, this specific anti-Nef sdAb could now help to the development of new antiviral strategies able to block HIV-1 replication; it will also constitute an efficient tool to elucidate the molecular functions of Nef in the virus life cycle.