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Selection of a macaque scFv with human-like framework regions, nanomolar affinity, and that neutralizes the lethal factor (LF) of *Bacillus anthracis in vitro* and *in vivo*, by inhibiting the PA/LF complex formation.

An anti-LF (B. anthracis) neutralizing macaque scFv

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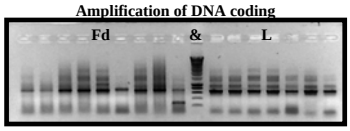
ABSTRACT :

Toxins necessary for Bacillus anthracis pathogenesis are made of three sub-units : PA (protective antigen), LF (lethal factor) and EF (edema factor). Anti-PA recombinant antibodies have been developed for anthrax treatment, but other sub-units have not been targeted despite anthrax experts recommendations. Here, we describe an anti-LF scFv that was obtained from a macaque (Macaca fascicularis) immune antibody gene library ($1.8x10^8$ clones) in pHAL14 phagemid vector. One scFv clone (2LF) selected from the library, had a high-affinity (KD = 1.02 nM), was highly neutralizing in the standardized in vitro (IC50 = 1.17 ± 0.06 nM) and in an in vivo assays. The genes encoding 2LF are similar to human immunoglobulin germline genes, and assigned to subgroups of human V, (D) or J genes by IMGT/V-QUEST. 2LF framework regions have a 84% identity with their most similar, germinally encoded human counterparts. This scFv neutralizes the anthrax lethal toxin by inhibiting the formation of the LF-PA complex, as shown in a competition assay. This inhibition suggests that 2LF interacts with domain 1 of LF, which is partially shared with EF and 2LF also reacted with EF, in ELISA and SPR. A 2LF-derived IgG, targeting LF and maybe EF, would be suitable for medical use.

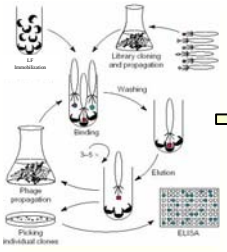
MATERIAL AND METHODS : Immunization of a macaque (M. fascicularis) with LF, construction and successful screening of the library.

IMMUNIZATION



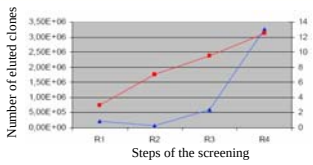
Bone marrow RNA extraction RT-PCR

Construction and screening of a library (10^8 clones) in pHal14.



Screening of the library was performed by phage display technology and 5 then 10, 20 and 40 washes were realized for each successive round of panning (see Laffly *et al*, *Antimicrob Agents Chemother* **49**:3414-20; 2005.).

Results of the screening



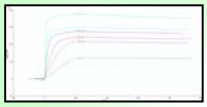
Steps of the screening

a) Eluted Phage during the screening (in blue); b) Results of Elisa-phage (in red), results obtained by DO measurement at 405nm for each sample divided by measure obtained for the negative witness (KLH).

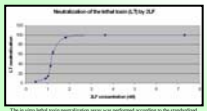
A 15-fold increase in the number of eluted phages between the first and the last previous round, and a 4-fold increase in the phage-ELISA signal obtained with those phages indicated an enrichment in phages reacting specifically with LF.

RESULTS : Selection of a scFv (2LF) reacting with LF (and EF), and neutralizing the anthrax lethal toxin

Best scFv (2LF) properties



SPR sensorgrams to evaluate 2LF/LF interaction.



The in vitro lethal toxin neutralization assay was performed according to the standardized protocol (**Little et al, *Infect Immun* 58: 1606-13; 1990.**)

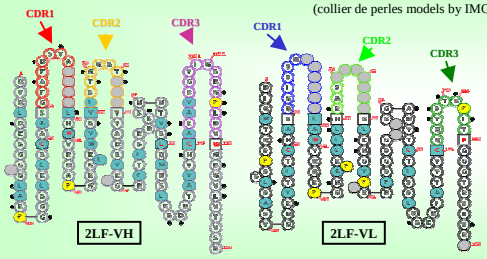
✓High affinity of 2LF for LF : **K_D = 1.02 nM** (by SPR)

✓2LF efficiently inhibited the toxicity of the lethal toxin in an *in vitro* **(CI50 = 1.20 nM),** and in an *in vivo* assay.

✓ ***In vivo***, 4/4 rats having received 12 µg of 2LF died, when none having received 24 µg. 2/4 rats were protected with 18 µg of 2LF.

scFv 2LF SEQUENCES

(collier de perles models by IMGT)



(Dots indicate differences from the human genes most similar to 2LF, and 2LF)

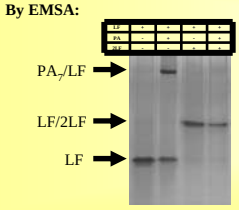
human germinal genes most similar to 2LF-encoding genes:

	V	D	J
VH	IGHV3-30*04	IGHD6-19*01	IGHJ3*01
VL	IGKV1-12*01		IGKJ1*01

→ **The overall identity between 2LF eight framework regions and their most similar human counterparts, coded by germinal genes, is 84.2%.**

Mechanism for lethal toxin inhibition : competition between 2LF and PA for LF binding

By EMSA:

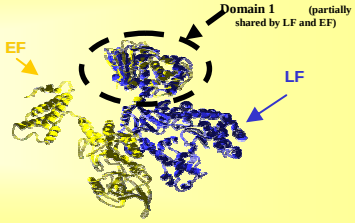


When PA and LF were incubated **in presence of 2LF**, 2LF-LF complexes only were visible, and **PA and LF did not combine**.

Identical result obtained with and in an equivalent competitive ELISA assay.

→ **The epitope of 2LF should thus be located in domain 1 of LF, which is involved in LF/PA interaction and is partially shared with EF.**

2LF reactivity with EF was positively tested in ELISA and SPR, and shown to have a **K_D = 5 nM**



PERSPECTIVES :

The strategy proposed here is the targeting of a bacterial toxin implied in immune evasion of a bacterial pathogen. This is a major role of the bacterial proteins broadly designated as virulence factors, and targeting virulence factors could be of wide interest. For instance, in animal models, the targeting by murine monoclonals of F1 and LcrV, two major *Yersinia pestis* virulence factors, has proved to be protective. Beyond bioweapons, targeting virulence factors of bacteria that pose a threat to public health could be envisioned and macaque antibody fragments may also be enlisted in such more civilian combats.