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

**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 (1.8x10⁹ 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 assay. 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.

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

**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.