Evaluating the antigenicity and stability of a hybrid molecule of pneumococcal surface protein A (PspA) and genetically detoxified pneumolysin (PdT) using molecular linkers

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Introduction

Pneumococcal protein vaccines have been proposed as better serotype-independent alternatives to the already used polysaccharide-based vaccines in order to increase coverage. PspA and PdT were genetically fused and protected mice against lethal challenge, offered higher cross protection against different strains and showed greater opsonophagocytosis rate than co-administered proteins. The first strategy used was the fusion *in tandem*, however it was unstable, which impaired purification scaling-up. In order to increase stability, antigens were fused with a rigid and a flexible molecular linker (PspA-RL-PdT and PspA-FL-PdT, respectively) between them and a C-terminal 6xHis-tag. This work aimed to produce stable hybrid molecules and evaluate their antigenicity.

Methods

The two constructs were cloned into *Escherichia coli* and clones were cultivated in defined culture medium. Hybrids were purified from soluble fraction using immobilized metal (Ni^{2+}) affinity chromatography and size exclusion chromatography. Stability was evaluated by SDS-PAGE and Western blot. Groups of BALB/c mice were immunized with 3 doses of each protein, and challenged intranasally three weeks after final dose, according to experimental design in Figure 1. Groups and assay details are described in Table 1. Antibody (total IgG) production was evaluated by ELISA.

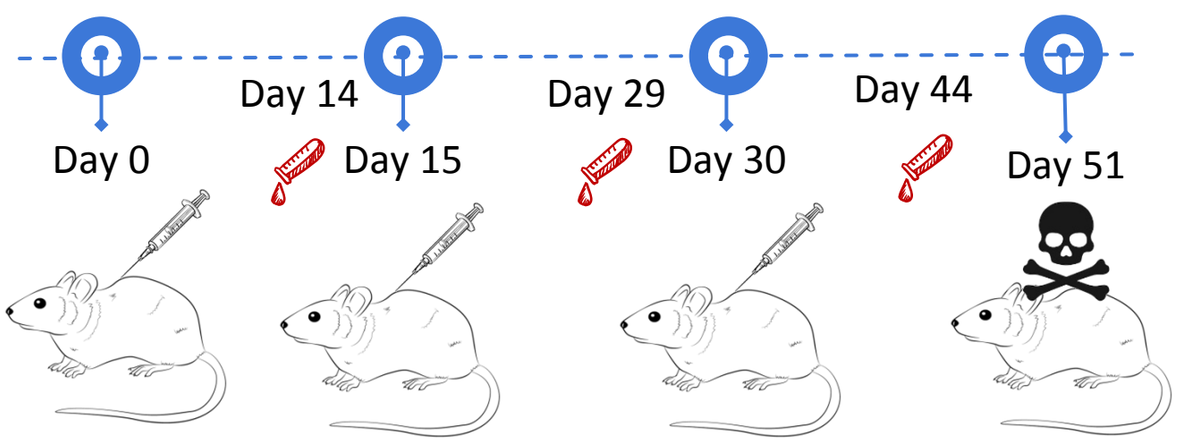

Figure 1. Experimental design of mice immunization and challenge. Protocol approved by the Ethic Committee on Animal Use of the Butantan Institute (CEUAIB) under protocol number 1905090218.

Mice	6 weeks female BALB/c	
Groups	PspA-FL-PdT 5 µg	PspA-RL-PdT 5 µg
	PspA-FL-PdT 10 µg	PspA-RL-PdT 10 µg
	PspA-FL-PdT 20 µg	PspA-RL-PdT 20 µg
	Sterile saline 0,9% + adjuvant	
Immunization via	Subcutaneous	
Adjuvant	Alum - 50 µg per dose of $Al(OH)_3$	
Lethal challenge	$1x10^5$ CFU of *S. pneumoniae* A66.1 Intranasally, 50 µL, anesthetized	

Table 1. Description of the mice groups and assay details.

Results

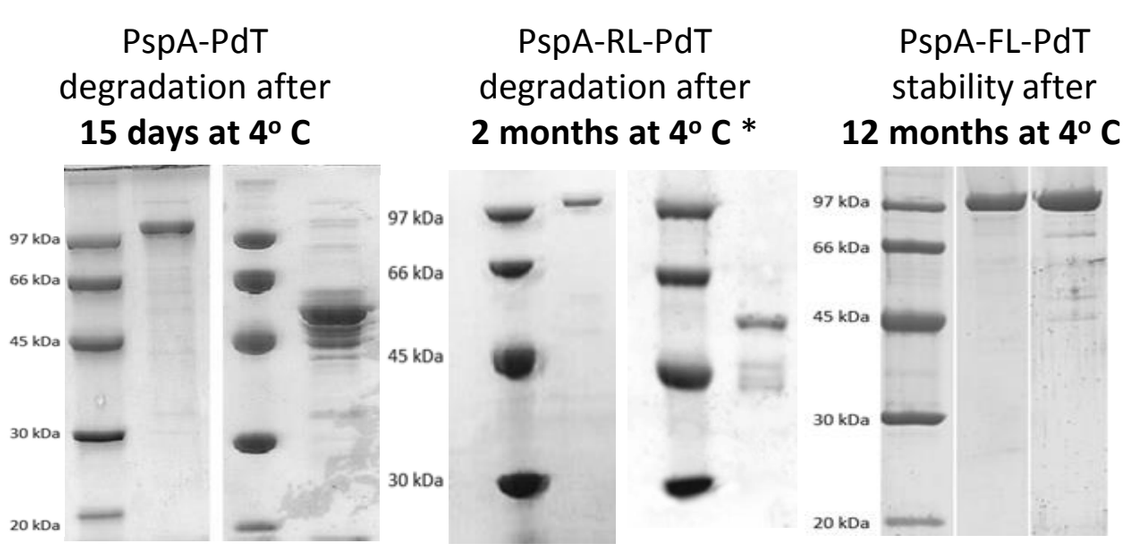

Figure 2. SDS-PAGE showing different purified hybrid molecules of PspA and PdT and their stability at 4°C. * PspA-RL-PdT and PspA-FL-PdT both remained stable for at least 12 months at -20°C.

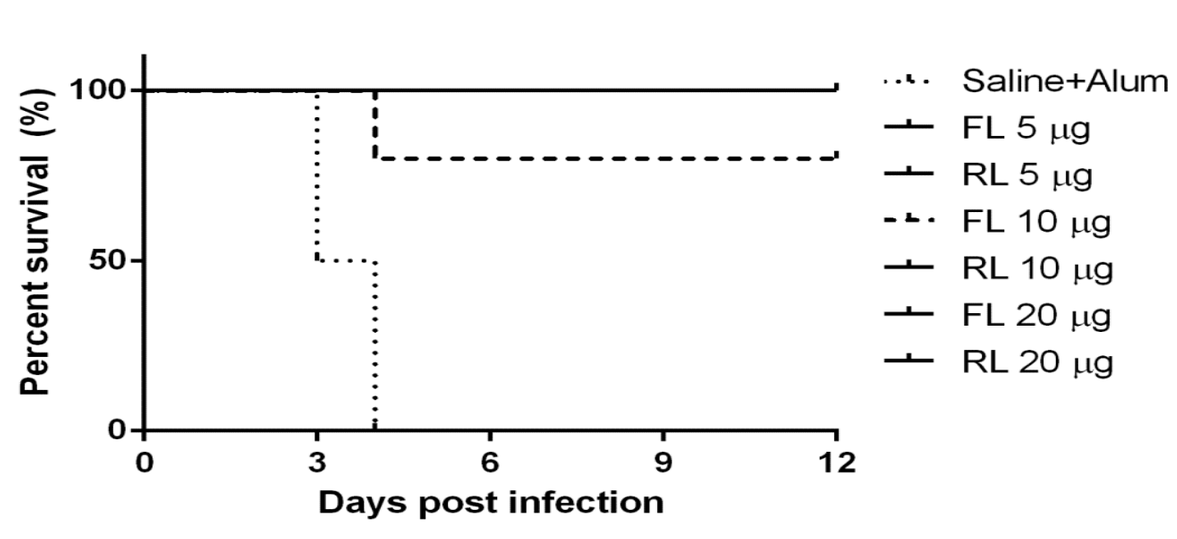

Figure 3. Survival curve after lethal challenge with *S. pneumoniae* strain A66.1. Mice were challenged with 1×10^5 CFU intranasally 21 days after the last dose. Statistical analysis by Log-rank Test (Mantel-Cox) – statistically, all treated animals survived the lethal challenge ($p<0.01$).

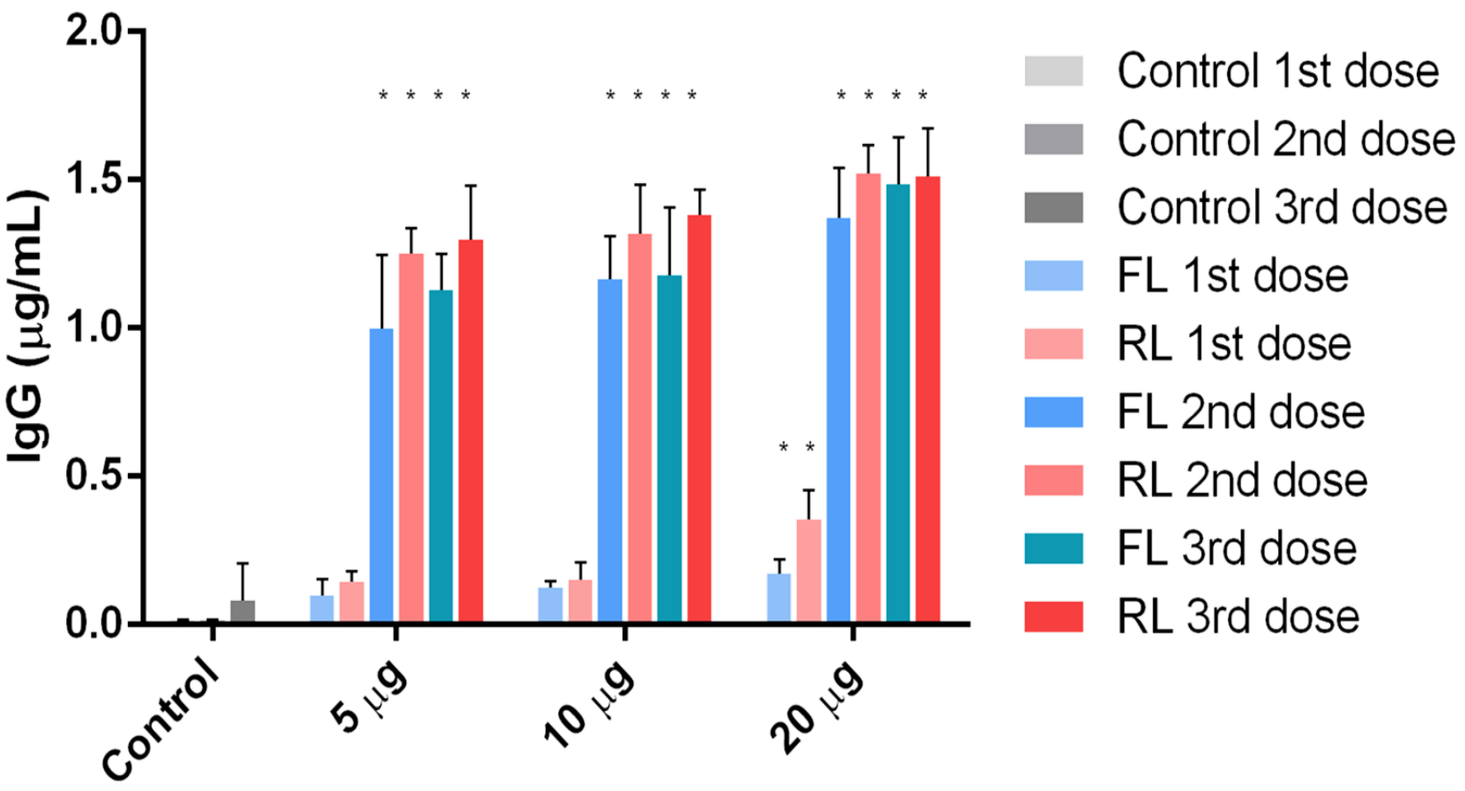

Figure 4. Antibody concentration of mice immunized with 3 doses of different masses of each protein. IgG concentration was measured 14 days after each dose and sera are diluted 16,000 times. Statistically significant differences are indicated (One-way ANOVA, Tukey's Multiple Comparison Test). * $p<0.001$ in comparison with control group.

- Molecular linkers increased the stability of the hybrid molecules, however it is not clear yet how hybrids are folded.
- Antibody levels measured after third dose were not statistically different of second dose in all groups.
- Since FL 5 µg and RL 5 µg groups were protected as much as the others, it can be inferred that the antibody concentration for each protein obtained after third dose in these groups was sufficient to generate protection. Similar antibody concentration was obtained for 10 µg of each protein after the second dose; therefore, only two doses of 10 µg would be probably sufficient to protect mice.
- Further immunization assay and structure analyses will be performed to evaluate previous hypotheses.

Support

CNPq FAPESP CAPES INSTITUTO BUTANTAN