Therapeutic effects of an antibody-derived peptide and its alanine scanning derivatives in a *Galleria mellonella* model of systemic candidiasis

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Background

The synthetic peptide T11F, with sequence identical to a fragment of the constant region of human IgM, and most of its substituted alanine derivatives proved to possess a significant *in vitro* candidacidal activity with half maximal effective concentrations in the micromolar range (Polonelli L et al. PLoS ONE. 2012;7(3):e34105). Selected peptides were also evaluated in the *Galleria mellonella* model of systemic candidiasis. A single injection of F11A derivative in larvae infected with *C. albicans* led to a significant increase in survival, as assessed by Mantel-Cox logrank test (** $p < 0.001$).

In vitro activity

asd ¹	Sequence	anti-Candida activity	
		EC ₅₀ ² (confidence intervals 95%) [mol/L] × 10 ⁻⁶	EC ₅₀ asd/EC ₅₀ T11F
T1A	ACRVDHRGLTF	0,738 (0,711–0,766) [§]	0,48
C2A	TARVDHRGLTF	32,521 (25,220–41,944) [§]	21,12
R3A	TCADVHRGLTF	13,458 (12,146–14,909) [§]	8,74
V4A	TCRADHRGLTF	1,134 (1,083–1,188) [§]	0,74
D5A	TCRVAHRGLTF	0,395 (0,389–0,402) [§]	0,26
H6A	TCRVDARGLTF	10,417 (9,948–10,901) [§]	6,76
R7A	TCRVDHAGLTF	12,055 (11,498–12,646) [§]	7,83
G8A	TCRVDHRALTF	1,551 (1,465–1,642)	1,01
L9A	TCRVDHRGATF	1,753 (1,679–1,830) [§]	1,14
T10A	TCRVDHRGLAF	0,850 (0,844–0,854) [§]	0,55
F11A	TCRVDHRGLTA	2,519 (2,433–2,607) [§]	1,64

¹asd, alanine-substituted derivative; ²EC₅₀, half maximal effective concentration; [§]significantly different activity compared T11F.

The most active
in vitro

The most active
in vivo

In vivo activity

In this study, T11F and the two selected derivatives, D5A, the most active *in vitro*, and F11A, showing *in vivo* efficacy were deeply investigated in *G. mellonella* to understand the possible mechanism of action of the therapeutic efficacy.

Materials and Methods

Last instar *G. mellonella* larvae (16 larvae/group) were used for this study. For toxicity experiments, larvae were inoculated directly into the hemocoel with the selected peptides (15 µmol/kg). Potential therapeutic activity of selected peptides was then evaluated by inoculating *C. albicans* SC5314 (5 × 10⁵ cells/larva) and the selected peptides (10 µmol/kg) or saline (control) 30 min after *Candida* challenge. Peptide modulation of host immunity to *C. albicans* (CA) infection was determined using hemocyte analysis, by hemocytometer count and cytospin, and larval histology.

Results and Discussion

All the tested peptides showed lack of toxicity in this experimental model, with survival curves similar to control group.

Cytology

Hemolymph analysis highlighted a different cell immune stimulation by the studied peptides. In particular, F11A was the most efficient in plasmatocyte and granulocyte activation.

Histology

F11A was the most active in eliciting nodule formation and melanization and in fat body activation, leading to a better control of yeast growth.

Overall, the different therapeutic effects observed *in vivo* against *C. albicans* systemic infection could be explained by *G. mellonella* immune system activation. Although all peptides displayed similar activity *in vitro*, only F11A efficiently increased larvae survival, by promoting a protective immune response characterized mainly by granulocytes and plasmatocytes. Our data suggest for F11A a double role, targeting simultaneously the fungus and the immune system, which results in pathogen clearance.