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Poster Abstract – E.19

CHARACTERIZATION OF ANTIFUNGAL RECOMBINANT ANTIBODIES EXPRESSED IN PLANTS

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Fungal diseases caused by opportunistic fungal agents are dramatically increased in the recent years, in particular candidiasis and aspergillosis carry high morbility and mortality in immunocompromised hosts. In a recent work [1] a specific anti-ß-glucan monoclonal IgG antibody, named 2G8, was generated in mice. This mAb bound the cell surface of several ß-glucan possessing pathogenic fungi, was able to directly inhibit fungal growth *in vitro* and exerted a protective effect *in vivo* against experimental systemic aspergillosis and cryptococcosis as well as against disseminated and vaginal infections by *Candida albicans*.

Gene engineering has been performed on sequences encoding variable regions of this mAb to devise novel anti-ß-glucan antibodies in different formats: a simple scFv antibody, a human chimeric antibody and a scFv-Fc miniantibody.

In order to exploit the potential of plants as ideal cost-effective expression system, the two full-length antibodies (human chimeric and scFv-Fc) have been transiently expressed in *Nicotiana benthamiana* plants by the vacuum-agroinfiltration technique. Western analysis of agroinfiltrated plant extracts revealed high expression levels of both chimeric light and heavy chains and of scFv-Fc. The full antibody and the scFv-Fc were purified from leaves by protein A affinity chromatography with very high yields (40 and 50 mg /kg of plant tissue, respectively). The correct assembly of both purified antibodies was evaluated by gel filtration and non reducing SDS-PAGE analysis. In addition, the scFv format expressed only in *E. coli* periplasm and purified by IMAC, retained the binding specificity of 2G8 mAb in both immunofluorescence and ELISA assays.

All engineered antibodies bound *C. albicans* and *A. fumigatus* hyphae. Similarly to what previously found for the protective murine mAb, the human chimeric mAb and the scFv-Fc preferentially recognized ß-glucan molecules in ß-(1,3) configuration and were able to inhibit growth of *C. albicans in vitro*, in the absence of immune effectors while preventing fungal adhesion to human epithelial cells.

In conclusion, we have generated a panel of recombinant antibodies, derived from a murine protective anti-ß-glucan monoclonal antibody. All recombinant antibody formats retain binding specificity and protection-relevant biological properties of the original mAb and, therefore, could represent potentially valuable tools for both diagnostics and immunoprophylaxis/therapy of human fungal diseases. The high yield obtained in plants confirm the notion that plants represent ultimate bioreactors for the expression of fully functional monoclonal antibodies.

References

[1] Torosantucci et al., A novel glycoconjugate vaccine against fungal pathogens, *J Exp Med* **202** (2005),597-606.