

### Recombinant Lectin *rML*: A Novel Inducer of Cytokines In Vitro

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The recombinant mistletoe lectin *rML* is a new biological entity being developed for cancer immunotherapy. *rML* is expressed in high yield in *Escherichia coli* and has affinity for galactopyranosyl residues. It consists of an A chain linked by a disulphide bridge to a B chain. The molecular weight of the heterodimeric protein is approximately 60 kDa. The B chain represents the carbohydrate binding part of the hololysin. The A chain enzymatically and irreversibly inactivates 28S ribosomal protein biosynthesis. The objective of this study was to analyze, firstly, the putative direct cytotoxic action of the bifunctional molecule *rML* against cancer cells and, secondly, its putative stimulating potency on human peripheral blood mononuclear cells (PBMC) and on the skin immune system which can initiate immune signaling cascades and responses against cancer cells. Cytotoxic action of *rML* was tested against the human lymphoblastic leukaemia cell line MOLT-4. *rML* eliminated half of the cancer cells at a concentration of about 30 pg/ml. The cytotoxic action of *rML* was inhibited by D-galactose,  $\beta$ -lactose and N-acetyl-D-galactosamine, respectively, and was accompanied by apoptotic processes. Quantitation of cells with apoptotic nuclei was performed morphologically by fluorescent microscopy after DAPI staining. Immune response modifying potency of *rML* was quantified, firstly, in an in vitro human skin<sup>®</sup> bioassay and in the HaCaT keratinocyte cell line culture system, respectively. The skin<sup>®</sup> bioassay consists of a three-dimensional fibroblast dermis and a structured epidermis of non-horned keratinocytes in their own naturally secreted matrix, all of human origin. *rML* concentration-dependently enhanced the release of IL-1 $\alpha$  and IL-6 from these skin cells. Secondly, *rML* concentration-dependently increased the release of IFN- $\gamma$  and TNF- $\alpha$  from PBMC. In conclusion, these observations altogether indicate that the multicytokine inducer *rML* is an immune response-modifying agent with an additional potent direct cytotoxic action against cancer cells.

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