

Plant-derived Murine IL-12 and Ricin B-Murine IL-12 Fusions

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Abstract

Interleukin-12 (IL-12), an important immuno-modulator for cell-mediated immunity, shows significant potential as a vaccine adjuvant and anti-cancer therapeutic. However, its clinical application is limited by lack of an effective bioproduction system and by toxicity associated with systemic administration of IL-12. The goals of this research were to determine whether plants can serve as an effective production system for bioactive IL-12, a complex 70 kDa glycoprotein cytokine, and whether the plant lectin RTB can facilitate mucosal delivery of IL-12 to immune responsive sites. Transgenic tobacco plants expressing murine IL-12 were generated and characterized. To ensure stoichiometric expression of the two separately encoded, disulfide-linked subunits of IL-12 (p35 and p40), a single-chain form of mouse IL-12 (mIL-12) was utilized. Hairy root cultures, as a fast-growing bioproduction system were developed from high expressers of mIL-12. A purification scheme was developed to purify plant-derived mIL-12 from hairy roots and purified mIL-12 was used to assess IL-12 bioactivity *in vitro* in mouse splenocytes and *in vivo* in mouse intranasal vaccination trials. Plant-derived mIL-12 triggered induction of interferon-gamma secretion from mouse splenocytes as well as stimulation of cell proliferation with comparable activities to those observed for the animal-cell-derived mIL-12. Mouse vaccination trials using green fluorescent protein (GFP) as the antigen and cholera toxin (CT) as the adjuvant suggested that plant-derived mIL-12 enhanced Th1 immunity and exhibited similar activity to animal-cell-derived mIL-12 *in vivo*. Plant-derived IL-12 itself was non-immunogenic suggesting conformational equivalency to endogenous mouse IL-12.

Ricin B (RTB), the non-toxic carbohydrate-binding subunit of ricin, directs uptake of ricin into mammalian cells and the intracellular trafficking of ricin A, the catalytic subunit of ricin. RTB's function suggests that it may work as a molecular carrier for effective mucosal delivery of IL-12. To prove this hypothesis, transgenic plants producing RTB:IL-12 fusions were generated and characterized. Our results demonstrated that RTB fused to the carboxyl-terminus of IL-12 maintained full lectin activity and IL-12 bioactivity. RTB fused to the amino-terminus of IL-12 did not show lectin activity perhaps due to steric hindrance. Purified IL-12:RTB from transgenic plant tissue was tested in an *in vitro* mucosal-associated lymphoid tissue (MALT) assay. The results indicate that RTB facilitates the binding of IL-12 to the epithelial cells and presentation of IL-12 to immune responsive cells.

In conclusion, this dissertation research has shown that transgenic plants are capable of producing valuable bioactive proteins, such as IL-12. Plant-derived mIL-12 exhibited similar activity to animal-cell-derived mIL-12 both *in vitro* and *in vivo*. Fusion of IL-12 with the RTB lectin facilitated the delivery of IL-12 to mucosal immune responsive cells and thus may serve as a molecular carrier to enhance IL-12 efficacy and reduce the side-effects associated with systemic administration of IL-12.

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List of Abbreviations

2-ME:	β - mercaptoethanol
acdIL-12:	Animal-cell-derived murine interleukin-12
ADP:	Adenosine diphosphate
APC:	Antigen-presenting cells
cGMP:	Current good manufacturing practice
CHO:	Chinese hamster ovary
CMI:	Cell-mediated immunity
CT:	Cholera toxin
CTB:	Cholera toxin B subunit
CTL:	Cytotoxic T lymphocytes
DMSO:	Dimethyl sulfoxide
DTT:	Dithiothreitol
ELISA:	Enzyme-Linked ImmunoSorbent Assay
Endo H:	Endoglycosidase H
ER:	Endoplasmic reticulum
FACS:	Fluorescence-activated cell-sorting
FBS:	Fetal Bovine serum
GFP:	Green fluorescent protein
HBSS:	Hank's balanced salt solution
hIL-12:	Human interleukin-12
IFN- γ :	Interferon-gamma
Ig:	Immunoglobulin
ISCOMS:	Immune stimulating complexes
LT:	Heat-labile enterotoxin from <i>Escherichia coli</i> .
LTB:	B subunit of LT
mAb:	Monoclonal antibody
MALT:	Mucosa-associated lymphoid tissue
MHC:	Major histocompatibility complex
mIL-12:	Murine interleukin-12
MS:	Murashige & Skoog salt mixture
NK:	Natural killer cells
OVA:	Ovalbumin
PBMC:	Peripheral blood mononuclear cells
PBS:	Phosphate buffer solution
PBST:	PBS with 0.1% Tween 20
PCR:	Polymerase chain reaction

pdIL-12:	Plant-derived murine IL-12
PEG:	Polyethylene glycol
PHA:	Phytohaemagglutinin
PNGase F:	N-Glycosidase F
PVP:	Polyvinyl pyrrolidone
RIP:	Ribosome-Inactivating Protein
RTA:	Ricin A subunit
scIL-12:	Single chain IL-12
scmIL-12:	Single chain murine IL-12
TCR:	T cell receptor
Th:	T helper cells
Th1:	T helper cells type 1
Th2:	T helper cells type 2
TNF- α	Tumor necrosis factor-alpha
VLPs:	Virus-like particles