SELECTIVE MANIPULATION OF MICROGLIA USING NON-VIRAL TARGETED DNA DELIVERY

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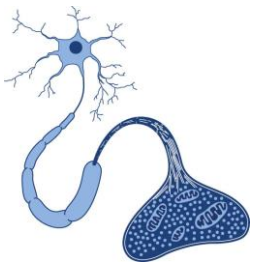

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THESIS SUMMARY

Microglia are the resident immunocompetent cells of the central nervous system (CNS) and are integrally involved in the response to disease and injury. There are currently limited approaches to investigate microglial function *in vivo*. Targeted delivery of DNA plasmids via cell surface receptors provides one possible means to selectively modify expression of proteins of interest. The potential of this approach has been demonstrated in previous studies using constructs termed "immunogenes" to selectively transfect neuronal subpopulations. These immunogenes consist of antibodies against a cell surface receptor linked to polycations, which in turn bind the DNA.

The broad aim of the present studies was to test whether immunogenes could be used to manipulate protein expression in microglia. The specific aims were to identify a receptor capable of supporting antibody internalisation into microglia, to generate immunogenes incorporating this antibody and test their capacity to transfect microglia in culture and *in vivo*.

Scavenger receptor class B, type I (SR-BI) was initially identified from published studies as a potential target for delivering DNA to glial cells. Using immunohistochemistry, SR-BI was identified in both astrocytes and microglia in mixed glial cultures. However, no immunolabelling of SR-BI was detected in adult

rat brain, despite the use of several antigen retrieval protocols. Antibodies directed against an external epitope of SR-BI were highly selectively internalised by microglia both in mixed glial cultures and in the adult brain. Uptake was not seen following injection of two other antibodies into the brain, suggesting that internalisation of this antibody either involved SR-BI that was present but not detected by immunohistochemistry, or resulted from interactions with another microglial protein. This result provided evidence of microglia-specific antibody delivery and prompted studies to test the capacity of immunogenes incorporating the SR-BI antibody to transfect microglia.

The initial immunogene was based on constructs used to successfully transfect neurons and contained poly-L-lysine as the polycation. The preparative procedure was subsequently extensively modified to improve immunogene recovery and increase DNA binding. Trials with the final construct resulted in transfection of occasional cells in culture and in the brain as detected from expression of green fluorescent protein encoded by the DNA plasmid. An alternative construct was developed incorporating polyethylenimine as the polycation. This construct produced transfection of both astrocytes and microglia in mixed glial cultures. Transfection efficiencies were much higher than with DNA bound to the polycation alone and similar to that achieved with other non-viral vectors.

More significantly, infusions of the immunogene into the hippocampus resulted in transfection of many cells, extending several millimetres from the infusion site. More than 80 % of transfected cells were immunoreactive for microglial markers. Transfection was not seen with an immunogene incorporating a different antibody or with DNA bound to free polyethylenimine. Transfection was also produced using single intracerebral injections of the SR-BI-based immunogene, albeit in fewer cells. Modifications of the immunogene were tested aimed at promoting release from intracellular vesicles and entry of DNA into the nucleus. These constructs also produced widespread selective transfection, but the modifications did not substantially increase transfection efficiencies.

These studies demonstrate that immunogenes can generate highly targeted transfection of microglia *in vivo* and constitute the first non-viral vector to achieve this outcome. The studies further identify the antibody against SR-BI as a novel carrier to selectively deliver DNA, and potentially other nucleic acids or drugs, to microglia in the brain. Further development of immunogenes or other constructs based on the SR-BI antibody has the potential to provide valuable means for better understanding the functions of microglia in the CNS.

DECLARATION

I declare that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any University; and that to the best of my knowledge and belief, it does not contain any material previously published or written by another person except where due reference is made in the text.

Josephine Malmevik, December 2012, Lund, Sweden

PUBLISHED ABSTRACTS ARISING FROM THIS WORK

Malmevik J.M.K., Herbert M.K., Berhanu D.A., Rogers M-L., Nilsson M., Nakanishi Y., Rush R.A., Sims N.R. and Muyderman H. *Scavenger receptor, class B, Type I: Expression in the adult rat brain and implications for receptor-mediated gene delivery.* POS-TUE-127. Australian Neuroscience Society (ANS) Annual Conference, Hobart, TAS, AU, 2008.

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Receptor, Class B, Type I. POS-MON-228. ANS Annual Conference, Sydney, NSW, AU, 2010.

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ABBREVIATIONS

Abbreviation	Explanation
A_{NNN}	absorbance at *NNN* nm
AAV	adeno-associated virus
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
amyloid-β	amyloid beta
AP	anteroposterior
ATP	adenosine-5'-triphosphate
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
bp / kbp	basepair / kilobasepair(s)
BSA	albumin from bovine serum
BSA/Triton IHC	immunohistochemistry using albumin from bovine serum as blocking agent
CA^N	*Cornu Ammonis N* field of the hippocampus
Ca^{2+}	calcium ion(s)
CD^N	cluster of differentiation molecule *N*
$CD206^{Ab}$	antibody against extracellular domain of CD206 mannose receptor
CHO	chinese hamster ovary
CL^N / CR^N CC^L / CCR^N $CXCL^N$ / $CXCR^N$	chemokine ligand / receptor cysteine-cysteine chemokine ligand / receptor chemokine ligand / receptor with two cysteines separated by one amino acid
Cl^-	chloride ion(s)
CMV	cytomegalovirus
CNS	central nervous system
Cre recombinase	causes recombination recombinase
Da / kDa	dalton(s) / kilodalton(s)
DMEM	Dulbecco's modified Eagle's medium (low glucose)
DNA	deoxyribonucleic acid
DNAse	deoxyribonuclease
DTS	DNA-targeting sequence
DTT	DL-dithiothreitol
DV	dorsoventral
E_N	embryonic day *N*
EDTA	ethylenediaminetetraacetic acid
EMSA	electrophoretic mobility shift assay
FBS	foetal bovine serum
Fc	fragment crystallizable
GDNF	glial-derived neurotrophic factor

Abbreviation	Explanation
GFAP	glial fibrillary acidic protein
GFP	green fluorescent protein
GIBCO H_2O	UltraPure™ DNase/RNase-free distilled water
GM-CSF	granulocyte/macrophage colony-stimulating factor
H^+	hydrogen ion(s)
HA2	hemagglutinin 2
HBSS	Hank's buffered salt solution
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HDL	high-density lipoprotein
HSV	herpes simplex virus
Iba1	ionized calcium binding adaptor molecule 1
IFNγ	interferon gamma
IgG	immunoglobulin G
IHC	immunohistochemistry
IL-^{N}	interleukin *N*
L	lateral
LB	Luria-Bertani
LDL	low-density lipoprotein
LPS	lipopolysaccharide
loxP	locus of crossing (x) over P1
Mac-1	macrophage-1 antigen
MCP-1	monocyte, memory T lymphocytes and NK-cell specific chemoattractant
MHC	major histocompatibility complex
mRNA	messenger ribonucleic acid
MS	multiple sclerosis
MWCO	molecular weight cut-off
NaCl	sodium chloride
NFκβ	nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	nerve growth factor
NHS	normal horse serum
NHS/Triton IHC	immunohistochemistry using normal horse serum as blocking agent
NLS	nuclear localisation signal
NO	nitric oxide
NPC	nuclear pore complex
O/N	overnight
P_N	postnatal day *N*
p75^{NTR}	p75 neurotrophin receptor
p75^{NTRAb}	antibody against the extracellular domain of p75^{NTR}
PBS	phosphate-buffered saline
PEI	polyethylenimine
PEST	penicillin-streptomycin
PFA	paraformaldehyde
PI	propidium iodide

Abbreviation	Explanation
pK_a	acid dissocation constant
PLL	poly-L-lysine
RNA	ribonucleic acid
rpm	revolutions per minute
RT	room temperature
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
siRNA	small interfering RNA
SMPT	sulfosuccinimidyl 6-(α-methyl-α[2-pyridyldithio]-toluamido)hexanoate
SPDP	N-succinimidyl 3-(2-pyridyldithio)propionate
SR-BI	scavenger receptor, class B, type I
$SR\text{-}BI^{Ab}$	antibody against extracellular domain of SR-BI
SV40	simian virus 40
TBE	tris-borate-EDTA
TGF-β	transforming growth factor β
TNF-α	tumour necrosis factor alpha
Traut's reagent	2-iminothiolane hydrochloride
Tris	tris(hydroxymethyl)aminomethane
TrkA	tropomyosin-receptor-kinase A
w/w ratio	weight/weight ratio

LIST OF FIGURES AND TABLES