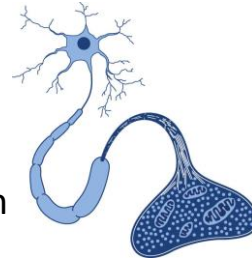


# SELECTIVE MANIPULATION OF MICROGLIA USING NON-VIRAL TARGETED DNA DELIVERY

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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.

A handwritten signature in black ink, appearing to read 'Josephine Malmevik', is written over a solid horizontal line.

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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# ABBREVIATIONS

| Abbreviation                      | Explanation  |
|-----------------------------------|--|
| $A_{NNN}$                         | absorbance at $NNN$ nm   |
| AAV                               | adeno-associated virus   |
| AD                                | Alzheimer's disease  |
| ALS                               | amyotrophic lateral sclerosis  |
| <i>amyloid-<math>\beta</math></i> | amyloid beta   |
| AP                                | anteroposterior  |
| ATP                               | adenosine-5'-triphosphate  |
| BBB                               | blood-brain barrier  |
| BDNF                              | brain-derived neurotrophic factor  |
| <i>bp / kbp</i>                   | basepair / kilobasepair(s)   |
| BSA                               | albumin from bovine serum  |
| BSA/Triton IHC                    | immunohistochemistry using albumin from bovine serum as blocking agent     |
| $CA^N$                            | <i>Cornu Ammonis N</i> 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  |
| $CL^N / CR^N$                     | chemokine ligand / receptor  |
| $CC^L / CCR^N$                    | cysteine-cysteine chemokine ligand / receptor                              |
| $CXCL^N / CXCR^N$                 | chemokine ligand / receptor with two cysteines separated by one amino acid |
| $Cl$                              | chloride ion(s)  |
| CMV                               | cytomegalovirus  |
| CNS                               | central nervous system   |
| <i>Cre recombinase</i>            | causes recombination recombinase   |
| <i>Da / kDa</i>                   | 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  |

## ABBREVIATIONS

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

## ABBREVIATIONS

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

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