Transfection of the cell lines COS-7 and P815 using Metafectene

Anna Hegele and Rainer Ulrich, Institut für Virologie, Universitätsklinikum Charité, Campus Mitte, Humboldt Universität Berlin, D-10098 Berlin

The objective of our investigations is the characterisation of the immune response induced by recombinant hantavirus proteins, which are potential vaccines against hantavirus infections. For that purpose BALB/c mice will be immunised with recombinant hantavirus proteins and the humoral and cellular immune response analysed.

For the detection of cytotoxic T cells, autologous antigen-presenting cells are needed. P815 cells (ATCC TIB-64) possess the same H-2^d haplotype as BALB/c mice. To generate stable transfectants, the entire nucleocapsid protein (N) encoding sequence of the Dobrava hantavirus (DOBV-Af prototype strain Slovenia; Avsic-Zupanc et al., 1995, J. Gen. Virol. 76, 2801-2808) was RT-PCR amplified, cloned into the plasmid pCR-TOPOII-Blunt (Invitrogen, Groningen, The Netherlands) and re-sequenced (pCR-TOPOII-Blunt-DOBV-N1b). Subsequently the correct sequence was sub-cloned into the plasmid pcDNA3 (Invitrogen; pcDNA3-DOBN#7).

To test whether the N protein can be expressed in mammalian cells, transient transfections were performed using cos7 cells (ATCC CRL-1651) kindly provided by Holger Loessner (Berlin). The cells were grown in RPMI medium containing 5% FCS and antibiotics. For transfection, the cells were seeded into 6-well-plates (approximately 5 x 10^4 cells per well). The transfections were performed according to the manufacturer´s protocol using transfection medium (RPMI medium containing 5% FCS, but without antibiotics). The transfection rate using Metafectene and Lipid E was estimated visually (Table 1). The number of cells in the field of view (magnification 40x10) was about 3600. Transfected cells were detected by an immunofluorescence assay on 12-well-slides using a hantavirus-N-specific monoclonal antibody 1C12 (Lundkvist et al., 1991, J. Gen. Virol. 72, 2097-2103), kindly provided by Åke Lundkvist (Stockholm), and FITC-labelled rabbit-anti-mouse-conjugate (DAKO Diagnostika, Hamburg, Germany).

Table 1
Transfection efficiency in COS-7 cells

Transfection reagent	Amount of DNA	Ratio Reagent : DNA	Number of positive cells	
			Absolute number	Relative amount
Metafectene	2 µg	7/1	appr. 200	5.5 %
	2 µg	5/1	appr. 1400	40 %
	2 µg	3/1	appr. 350	10 %
Lipid E*	0.4 µg	25/1	appr. 30	0.83 %

*The transfection protocol for Lipid E was not optimised in this experiment.

Transient transfections were performed using an adherent subline of P815 cells, kindly provided by Günther Schönrich (Berlin). The cells were grown in DMEM medium containing 5% FCS, L-glutamine and antibiotics. Before transfection the cells were seeded into 6-well-plates (approximately 2,5 x 10^4 cells per well). The transfections were performed according to the protocol of the manufacturer in transfection medium (DMEM medium containing 5% FCS and L-glutamine, but without antibiotics). The transfection efficiency was estimated using a immunofluorescence assay as described above (Table 2). In this experiment Lipid L was used as a control transfection reagent because transfections using Lipid E revealed only a very low number of positive cells.

Table 2
Transfection efficiency in P815 cells

Transfection reagent	Amount of DNA	Ratio Reagent : DNA	Relative amount of positive cells
Metafectene	2 µg	7/1	0.5 – 1 %
	2 µg	5/1	1 %
	2 µg	3/1	5-10 %
Lipid L*	4 µg	2,5/1	0.1 – 0.5 %

*The transfection protocol for Lipid L was not optimised in this experiment.

Stable transfectants

To generate stable transfectants, the plasmid pBMGneo (Schirmbeck et al., 1994, J. Immunol. 152, 1110-1119) kindly provided by Reinhold Schirmbeck (Ulm) was used. The N protein-encoding sequence was isolated as an *Eco*32I/*Ecl*136II fragment from the plasmid pCR-TOPOII-Blunt-DOBV-N1b and subsequently inserted into *Sal*I-linearized, Klenow-polymerase treated plasmid pBMGneo. For transfection P815 cells were seeded in 6-well-plates one day before transfection (2.5×10^4 cells/well) using medium without antibiotics. Four hours before transfection the medium (without antibiotics) was exchanged. The transfection was performed according to the manufacturer´s protocol using a DNA/Metafectene ratio of 1:3 (2µg DNA + 6µl Metafectene). After about 72 hours the selection process was started by splitting the cells. The cells (100 cells per well) were seeded into 10 cm-plates (10 ml medium) and 0.25 mg/ml G418 (PAA Laboratories GmbH, Cölbe, Germany) was added. Every third or forth day the concentration of G418 was increased stepwise by 0.25 mg/ml until a final concentration of 1.5 mg/ml was reached. Single cell colonies were isolated and separately grown in 24-well-plates using a G418-concentration of 1 mg/ml. The detection of stable transfectants expressing the N protein of DOBV was obtained by immunofluorescence assay as described above.