Ex vivo labeling of primary human monocytes with ultra small particles of iron oxide using different transfection agents (methodology)

Oude Engberink RD, Van der Pol SMA*, Dopp E*, Renardel de Lavalette C*, De Vries HE*, Blezer ELA
Dept of Experimental in vivo NMR, Image Sciences Institute, University Medical Center Utrecht, *Dept of Molecular Cell Biology and Immunology, VU Medical Center, Amsterdam

Lesion formation in multiple sclerosis (MS) is characterized by infiltration of immune cells into the brain, ultimately leading to destruction of myelin sheets. Monocyte-derived macrophages play a crucial role in the neuropathogenesis of MS. Cell imaging by magnetic resonance (MR) may be a powerful tool to reveal migration in vivo and provide crucial information on lesion development longitudinally. For this purpose, cells have to be isolated, labeled ex vivo with MR contrast agents and reintroduced into the body.
In this study we aimed to develop an efficient ex vivo labeling method of primary human monocytes. As suitable contrast agents we used ultra small particles of iron oxide (USPIO's), because they are relatively small and have a large T2-effect. Two differently sized USPIO's (Sinerem: 30 nm and Endorem: 50-150 nm; 1mg Fe/ml medium) were used. We further explored whether pre-incubation with transfection agents (TA's; Poly-L, Fugene and Superfect) improved labeling efficiency. Uptake of iron was determined by T2-relaxation time MRI and Perls' stain for iron. The effect of labeling on activation and survival of the monocytes was verified by viability, migration and adhesion assays.
Incubation of monocytes with Sinerem showed no significant labeling. Pre-incubation of Sinerem with TA's did not change this result. In contrast, incubation of monocytes with Endorem resulted in a labeling efficiency higher than 80%. T2-relaxation time decreased up to 40%. Viability of the labeled cells decreased with 5%, migration and adhesion did not change as compared to control cells. Pre-incubation of Endorem with Superfect resulted in an increase of iron particles per cell.
Our data suggest that Endorem is the most suitable USPIO for labeling monocytes ex vivo. The use of Superfect enhances the uptake of Endorem. The ex vivo labeling procedure shows great potential for real-time tracking of monocytes in vivo with MRI.

Raoul D. Oude Engberink, Department of Experimental in vivo NMR, Image Sciences Institute, University Medical Center Utrecht, Bolognalaan 50, 3584 CJ Utrecht, t 030 253 5515, e-mail raoul@invivonmr.uu.nl

Postersessions: Neuroscience 1 on Wednesday 2 June
Neuroscience 2 on Thursday 3 June