Gene Function in Cell Growth, Differentiation &Development

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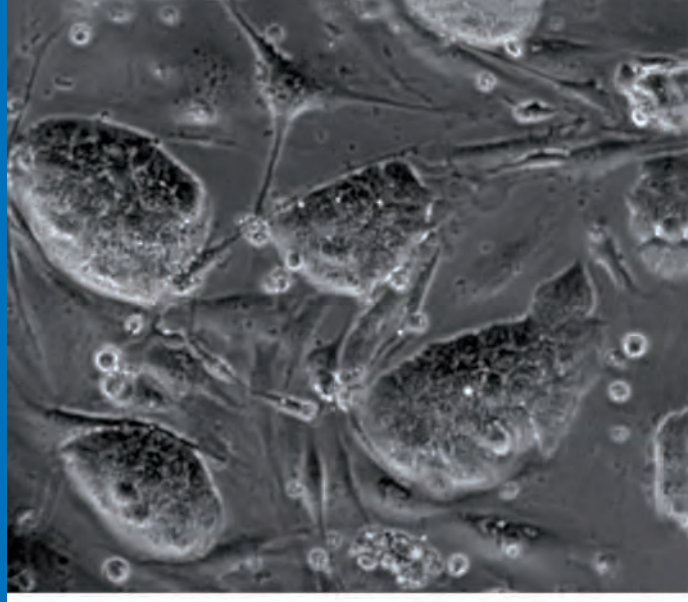

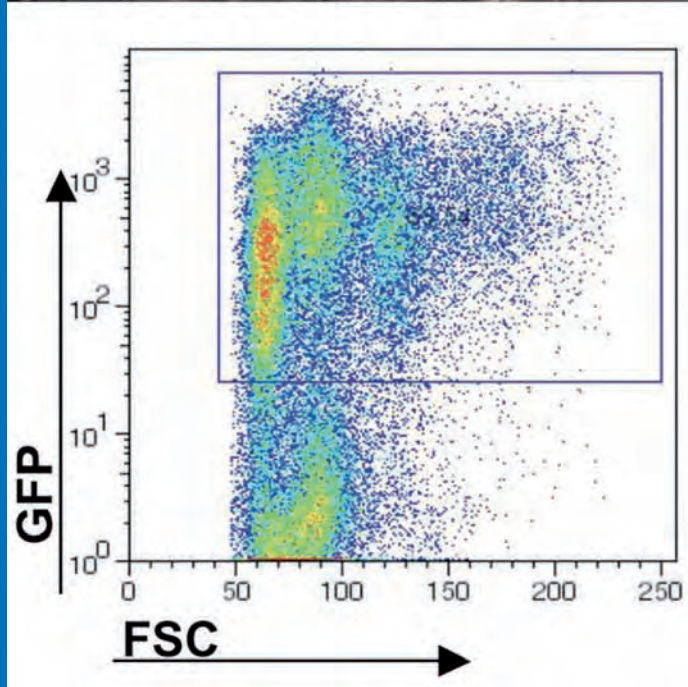

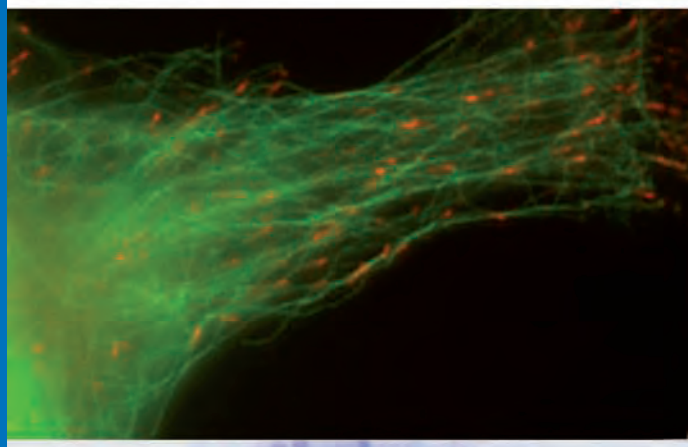

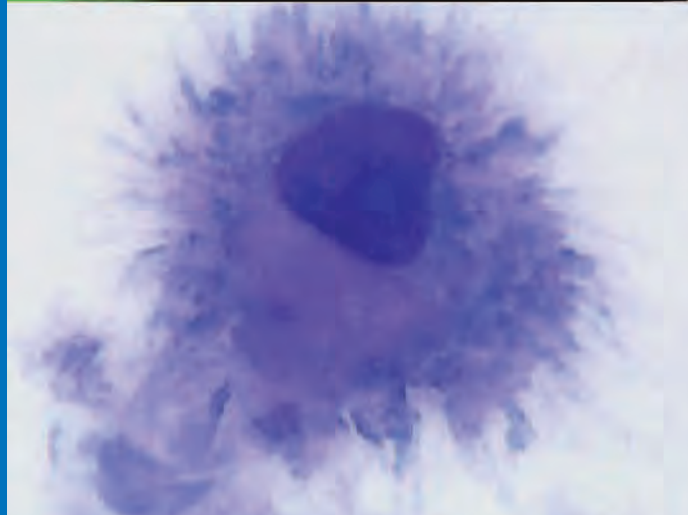

Introduction

Cell identity and function are determined by genetic programs, involving a multitude of regulatory circuitries and signalling pathways. Our research focuses on hematopoietic stem/progenitor cells and their differentiated progeny, and how cell fate and function are specified. Stem cells represent a particular attractive cell type for studying cell fate decisions, since they combine two unique properties in one cell: a high self-renewal activity and a broad differentiation potential, which puts stem cells aside from most other somatic cells.
Stem/progenitor cells are isolated from human cord blood, bone marrow or peripheral blood and from mouse bone marrow, and cells are grown with specific cytokines. Cells are then induced to differentiate with yet another set of cytokines and/or differentiation factors, and the ongoing changes in gene expression are monitored on a genome-wide scale by DNA microarrays. The development of antigen presenting dendritic cells (DC) and red blood cells is examined in detail. Mouse embryonic stem cells (ES cells) are also studied. Genes with a determining role in cell fate decisions and cell functions are analyzed in vitro and in vivo in knockdown approaches and knockout mouse models. Cells are transplanted into recipient mice and synthetic nano-sized particles are employed for monitoring cell position and function by magnetic resonance imaging (MRI) in vivo.
Biomedical engineering involves the development of biohybrid systems containing cells and bioengineered scaffolds. In this context we study the impact of natural and synthetic biomaterials on cell differentiation and function, including the induction of unwanted immune responses elicited by DC activation. Cells are endowed with novel and wanted properties by standard technology of genetic engineering and implanted in biohybrid systems for use in medical therapy.

Towards determining the genetic repertoire of hematopoietic stem cells

In multicellular organisms specific stem cell types with distinct developmental potentials occur during development. Transient pluripotent cells, which can differentiate into derivatives of all three germ layers (endoderm, ectoderm and mesoderm), are generated during blastocyst development. Adult stem cells, developing at later stages, are more restricted in their potential, since they can differentiate into progenitors and mature effector cell types of only one stem cell system. Adult stem cells have been identified in a variety of tissues in the adult organism and are important for lifelong tissue homeostasis and repair.

In our previous work we studied a c-kit$^+$ Flt3$^+$ CD11b$^+$ hematopoietic stem/progenitor cell from mouse bone marrow (referred to as Flt3$^+$ stem/progenitor cell) and its differentiation potential (Hacker et al., 2003; Hieronymus et al., 2005; 2008; Zenke and Hieronymus, 2006a; Figures 1-4). Such Flt3$^+$ stem/progenitor cells give rise to all mature blood cells and blood-borne cells in peripheral lymphoid organs following transplantation in vivo.

ES cells are pluripotent cells, which can differentiate into derivatives of all three germ layers (Figure 1). Pluripotency is

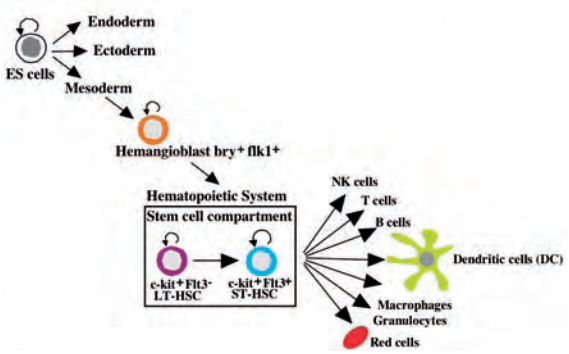

Figure 1: ES cells give rise to cells of all three germ layers (endoderm, ectoderm and mesoderm), including hematopoietic stem cells. Hematopoietic stem cells develop into all mature blood cells. The laboratory has a particular interest in DC and red cell development.

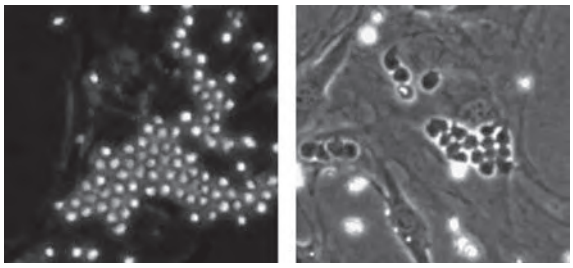

Figure 2: Coculture of Flt3$^+$ stem/progenitor cells on OP9 stroma cells (left), revealing "cobble stone area forming cells" that are located below OP9 stroma cells (right).

regulated by specialized regulatory circuitries, involving specific transcription factors and signalling pathways. Transcription factors represent a class of DNA binding and DNA associated proteins with a determining function in cell fate decisions and cell development. By employing gene expression profiling with DNA microarrays we study the transcription factor repertoire of Flt3$^+$ stem/progenitors and relate that to the transcription factor repertoire of ES cells (Figures 3 and 4; in collaboration with A. M. Müller, Julius-Maximilians-University, Würzburg, Germany; S. Rose-John, Christian-Albrechts-University, Kiel, Germany and A. M. Wobus, Institute of Plant Genetics and Crop Plant Research, IPK, Gatersleben, Germany). We found that hematopoietic stem cells and ES cells show overlapping and non-overlapping expression pattern of transcription factors and thus provide novel insights into the dynamic networks of transcriptional regulation in embryonic and adult stem cells (Hieronymus et al., 2008).

Cell fate decisions by reactivation of stem cell genes and pluripotency associated genes

DNA is embedded in a plethora of proteins, referred to as chromatin, that regulate DNA replication and gene transcription. In a previous study we observed that treatment of neural stem cells (Figure 5) with the chromatin modifying agents trichostatin A (TSA) and 5-Aza-2'-deoxycytidine (AzaC) enlarges their developmental potential and make them acquire hematopoietic activity in vivo (Schmittwolf et al., 2005; in collaboration with A. M. Müller, Julius-Maximilians-University,

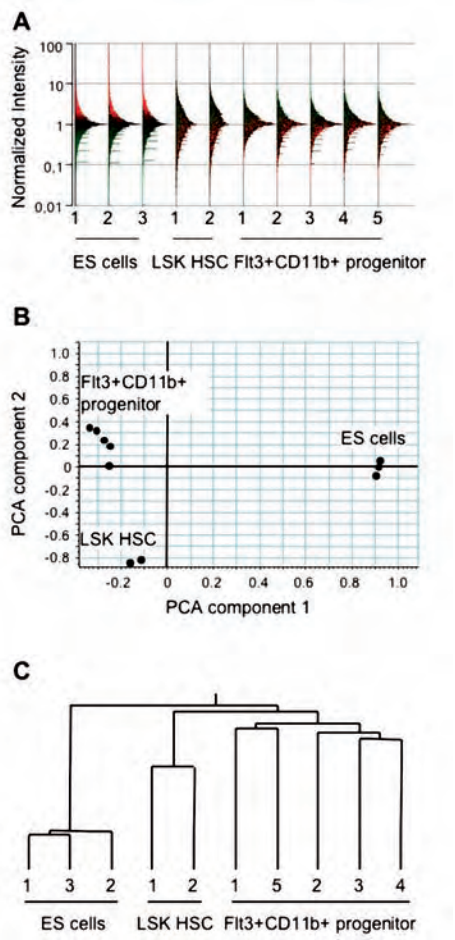

Figure 3: Microarray analysis of Flt3$^+$ hematopoietic stem/progenitor cells. Normalization of DNA microarray data, principal component analysis (PCA) and conditional tree cluster analysis (A, B and C, respectively) of Flt3$^+$ stem/progenitor cells, ES cells and lineage-negative Sca-1$^+$ c-kit$^+$ Flt3$^-$ hematopoietic stem cells (long-term (LT) reconstituting LSK HSC; Figure 1) (Hieronymus et al., 2008).

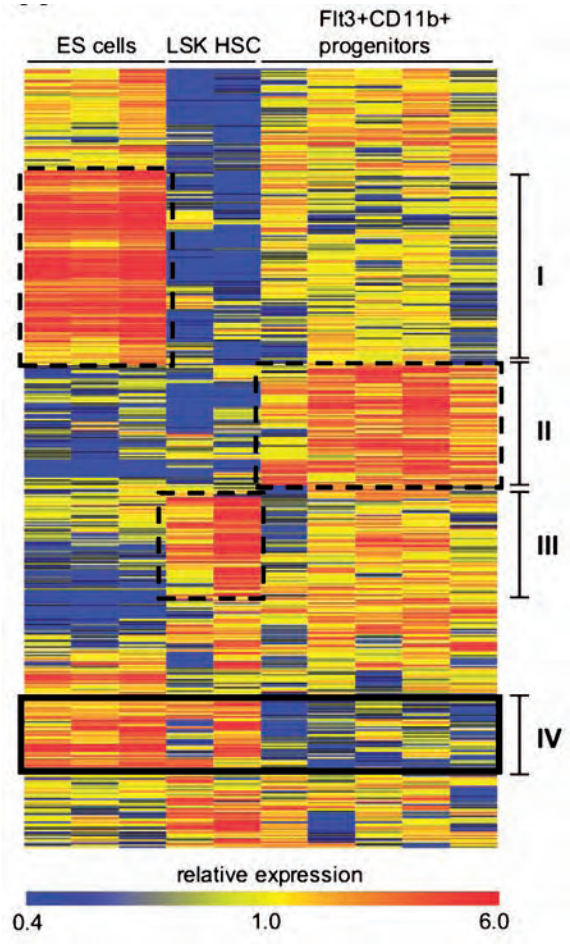

Figure 4: Hierarchical cluster analysis of transcription factors in Flt3$^+$ stem/progenitor cells, LSK HSC and ES cells (same cells as in Figure 3). The colour of the respective box in one row represents the expression value of the gene transcript in one sample. Blue, low expression; yellow, intermediate expression; red, high expression (Hieronymus et al., 2008).

Würzburg, Germany). The TSA/AzaC induced hematopoietic activity was long-term, multi-lineage and transplantable.

TSA and AzaC affect histone acetylation and DNA methylation, respectively, and thus chromatin architecture and gene expression. We have now analyzed the TSA/AzaC induced changes in gene expression by global gene expression profiling with DNA microarrays (Ruau, Ensenat-Waser et al., 2008; in collaboration with A. M. Müller, Julius-Maximilians-University, Würzburg, Germany and A. M. Wobus, Institute of Plant Genetics and Crop Plant Research, IPK, Gatersleben, Germany). TSA/AzaC caused both up- and down-regulation of genes without increasing the total number of expressed genes. Chromosome analysis showed no hotspot of TSA/AzaC impact on a particular chromosome or chromosomal region. Hierarchical cluster analysis revealed common gene expression pattern of neural stem cells treated with TSA/AzaC, ES cells and hematopoietic stem cells. Furthermore, our analysis identified several stem cell genes and pluripotency-associated genes that are induced by TSA/AzaC, including CD34, CD133, Oct4, Nanog, Klf4, Bex1 and the Dppa (developmental pluripotency associated) family members Dppa2, 3, 4 and 5 (Figure 6). Sox2 and c-Myc are constitutively expressed in neural stem cells. We propose a model where TSA/AzaC - by removal of epigenetic inhibition - induces the reactivation of several stem cell genes and pluripotency-associated genes, and their coordinate expression enlarges the differentiation potential of otherwise tissue restricted somatic cells.

Mouse and human somatic cells acquire pluripotency by the expression of a defined set of factors: Oct4, Sox2, c-Myc, and Klf4, referred to as induced pluripotent stem (iPS) cells (see Kim, Zaehres et al., 2008, for references). iPS cells can also be derived with only three of these factors (Oct4, Sox2, and Klf4). Mouse neural stem cells express Sox2 and also elevated level of c-Myc (Ruau, Ensenat-Waser et al., 2008; Kim, Zaehres et al., 2008). Thus, iPS cells were derived from neural stem cells by the expression of only Oct4 together with either Klf4 or c-Myc (Kim, Zaehres et al., 2008). These two-factor (2F) iPS cells contribute to development of the germline and form chimeras. Additionally, genome-wide gene expression profiling demonstrates that 2F iPS cells are similar to ES cells at the molecular level (Kim, Zaehres et al., 2008; in collaboration with H. R. Schöler, MPI for Molecular Biomedicine, Münster, Germany). Thus, endogenous expression of Sox2 in neural stem cells complements the 2F reprogramming process.

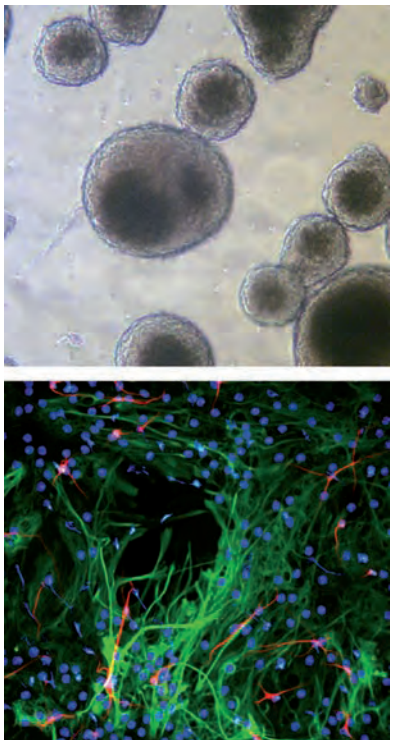

Figure 5: Neural stem cells form floating spheres (neurospheres) in culture (top) and differentiate into astroglial cells (GFAP, glial fibrilaric acidic protein, green) and neuronal cells (tubulin-beta-III, red) (bottom). Nuclei are stained with DAPI (blue) (Ruau, Ensenat-Waser et al., 2008).

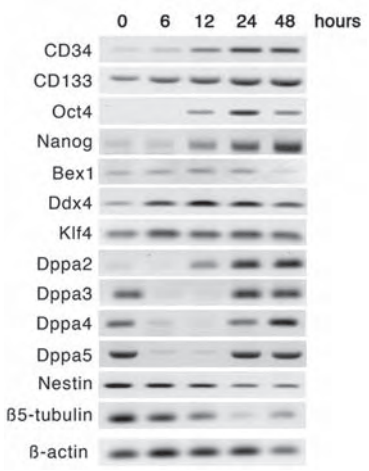

Figure 6: Kinetics of stem cell genes and pluripotency-associated genes during TSA/AzaC treatment of neural stem cells analyzed by RT-PCR. Loading control, β-actin (Ruau, Ensenat-Waser et al., 2008).

Assessing the interaction of stem cells with biomaterials

Embryonic and adult stem cells represent ideal instigators of regenerative processes. Yet, in many instances their use in cell-based therapies will require their application in biohybrid systems, where cells are seeded in biomaterial scaffolds. Such cell-biomaterial hybrids provide a microenvironment to ensure cell survival after transplantation.
To this end we have analyzed the interaction of a variety of stem cell types with a large panel of biomaterials (Neuss et al., 2008). This work was the concerted effort of various institutions at RWTH Aachen University, including W. Jahnen-Dechent, Institute for Biomedical Engineering, Biointerface Laboratory, The Interdisciplinary Centre for Clinical Research, IZKF "BIOMAT.", Institute of Pathology, Clinics of Conservative Dentistry, Periodontology and Preventive Dentistry, and Clinics of Plastic Surgery, Hand Surgery and Burn Unit, RWTH Aachen University Hospital (in collaboration with the Institute for Textile Chemistry and Macromolecular Chemistry, and German Wool Research Institute, RWTH Aachen University, Aachen, Germany and Institute of Clinical Genetics, Carl-Gustav-Carus University Hospital, Dresden, Germany).

Frequently, the identification and development of biomaterials is an iterative process where biomaterials are designed and then individually tested for their properties in combination with one specific cell type. However, recent efforts have been devoted to systematic, combinatorial and parallel approaches to identify biomaterials that are suitable for specific applications. Parameters such as surface topology and physicochemical properties, including surface wettability and surface charge, strongly influence cell-biomaterial interactions. Yet so far, due to the complex nature of such interactions, no general principles are known that allow a prediction of cell behaviour on a given biomaterial surface.

We have therefore used a grid-based platform for systematic assessment of stem cell-biomaterial interactions and (i) established a Biomaterial Bank of known and newly synthesized polymers and (ii) tested embryonic and adult stem cell types, including pluripotent ES cells and multipotent adult stem cells (mesenchymal stem cells, preadipocytes, dental pulp stem cells, hematopoietic stem cells and endothelial progenitor cells). Parameters such as cell morphology, adhesion and proliferation, vitality, cytotoxicity and apoptosis were systematically analyzed and this now allows to suggest and advise for or against a specific stem cell/biomaterial combination (Neuss et al., 2008).

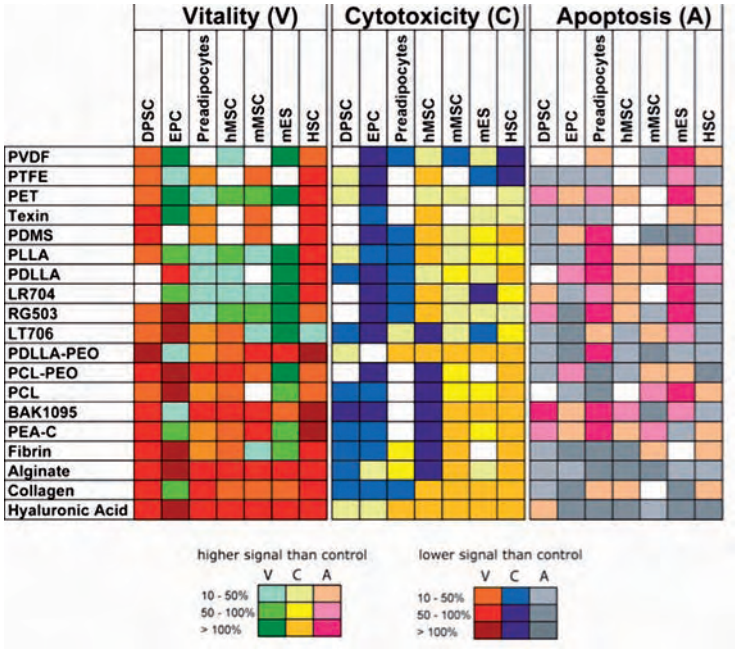

Figure 7: Multiplex assay of various stem cell types on polymers of the Biomaterial Bank; DPSC: dental pulp stem cells; EPC: endothelial progenitor cells; hMSC: human mesenchymal stem cells; mMSC: mouse mesenchymal stem cells; mES: mouse embryonic stem cells (Neuss et al., 2008).

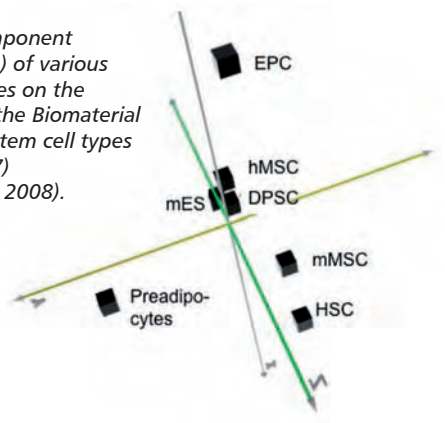

Figure 8: Principal component analysis (PCA) of various stem cell types on the polymers of the Biomaterial Bank (same stem cell types as in Figure 7) (Neuss et al., 2008).

The biomaterials of the Biomaterial Bank are also being used to assess their impact on immune cells, including antigen presenting dendritic cells (DC), that have a key role in immunity and tolerance induction (Zenke and Hieronymus, 2006b). Mouse knockout models, which are deficient in or lack specific DC subsets, are used to determine the role of DC in adverse reactions to biomaterials. These studies are expected to improve on the design and development of bioprostheses that do not trigger inflammatory or fibrotic responses.

Monitoring cell position and function in vivo

Following transplantation, cell position and function need to be monitored to serve as a quality control for successful cell therapy. To this end we are developing synthetic nano-sized particles, which functionally integrate into cells and subcellular structures, for use in magnetic resonance imaging (MRI) (Himmelreich et al., 2006; Becker et al., 2007; in collaboration with M. Hoehn, MPI for Neurological Research, Cologne, Germany; U. Himmelreich, Catholic University of Leuven, Belgium; S. Aime, University of Torino, Italy; W. Richtering, Institute for Physical Chemistry, M. Hodenius and T. Schmitz-Rode, Applied Medical Engineering, Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany).

DC represent professional antigen presenting cells (Zenke and Hieronymus, 2006b) that efficiently take-up antigens,

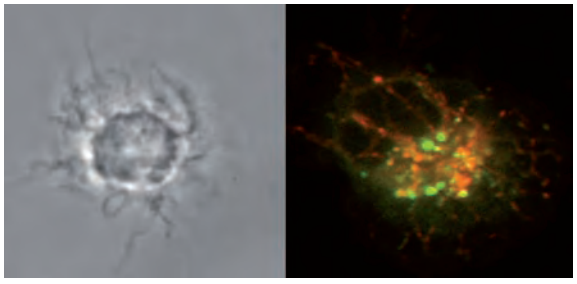

Figure 9: Magnetic iron oxide nanoparticles are taken-up by DC. Phase contrast image (left). Intracellular localization (right) of FITC-labeled iron oxide nanoparticles (green) and Lysotracker staining of the lysosomal compartment (red).

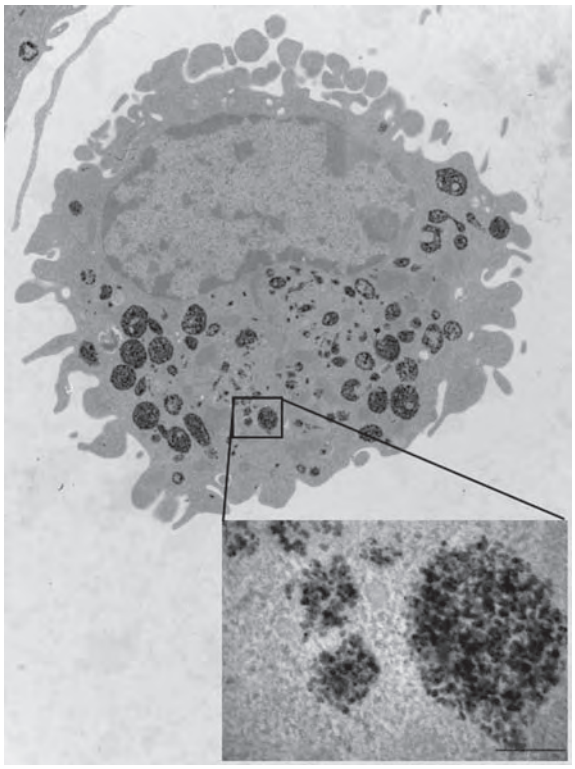

Figure 10: Electron micrograph of iron oxide nanoparticles in DC.

including nanoparticles, and thus can trigger unwanted immune responses. We thus investigate the impact of various formulations of iron oxide nanoparticles on DC function (Figures 9 and 10), aiming at the development of engineered nanoparticles for MRI that are immunologically inert.

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Further reading

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