J Neuroimmune Pharmacol (2015) 10:1–13
DOI 10.1007/s11481-015-9588-y

PERSPECTIVE

Nanomaterial Applications in Multiple Sclerosis Inflamed Brain

Clara Ballerini · Giovanni Baldi · Alessandra Aldinucci · Pietro Maggi

Received: 17 December 2014 / Accepted: 14 January 2015 / Published online: 24 January 2015

Abstract In the last years scientific progress in nanomaterials, where size and shape make the difference, has increased their utilization in medicine with the development of a promising new translational science: nanomedicine. Due to their surface and core biophysical properties, nanomaterials hold the promise for medical applications in central nervous system (CNS) diseases: inflammatory, degenerative and tumors. The present review is focused on nanomaterials at the neuro-immune interface, evaluating two aspects: the possible CNS inflammatory response induced by nanomaterials and the developments of nanomaterials to improve treatment and diagnosis of neuroinflammatory diseases, with a focus on multiple sclerosis (MS). Indeed, nanomedicine allows projecting new ways of drug delivery and novel techniques for CNS imaging. Despite the wide field of application in neurological diseases of nanomaterials, our topic here is to review the more recent development of nanomaterials that cross blood brain barrier (BBB) and reach specific target during CNS inflammatory diseases, a crucial strategy for CNS early diagnosis and drug delivery, indeed the main challenges of nanomedicine.

C. Ballerini (✉) · A. Aldinucci
Department of Neurofarba, University of Florence, Viale Pieraccini, 6, 50137 Florence, Italy
e-mail: clara.ballerini@unifi.it

G. Baldi
Ce.Ri.Col -Research Center Colorobbia, Montelupo Fiorentino, Florence, Italy

P. Maggi
Hôpital Erasme -Université libre de Bruxelles -Bruxelles-Be, Brussels, Belgium

P. Maggi
National Institute of Neurological Disorders and Stroke -NIH-Bethesda-US, Bethesda, MD, USA

Keywords Nanotechnology · Nanomaterials · Imaging · Therapy · Brain inflammation · Multiple sclerosis

Introduction

Inflammation in the CNS

The presence of blood brain barrier (BBB), an interface separating CNS from peripheral circulation, makes immune reaction in brain a very specific mechanism. BBB is determined, together with astrocytes and pericytes, by endothelial cells that form cell-cell tight junctions that physically restrict blood brain solute flow (physical barrier), limit passive diffusion of small lipophilic molecules, select active passage of hydrophilic compounds, transport proteins and exert a antitoxic activity through the action of metabolic enzymes (biochemical barrier) (Abbott et al. 2010). Several endogenous transporter of the BBB permit passage of nutrients and minerals, at the same time BBB limits passage of foreign molecules as drugs and diagnostic contrast medium. Despite this complicate anatomic organization and restricted immune cell access, CNS inflammation is not uncommon and is given by innate immune response mainly driven by astrocytes and microglia, resident CNS cells, upon pathogen exposure of innate immune receptors (Toll like receptors, TLRs and nucleotide binding domain leucine-rich repeat containing receptors, NLRs). Microglia and astrocytes activation determines inflammatory cytokine cascade, diminishing BBB barrier function with consequent increase of peripheral cells recruitment (Wraith and Nicholson 2012). This mechanism is not restricted to autoimmune or infectious diseases: where CNS is damaged TLRs and NLRs sense non-infectious tissue injury, nucleic acids and misfolded proteins, as it happens in trauma, stroke

or in chronic degenerative diseases and give rise to an inflammatory reaction (Zhang et al. 2010; Hanisch and Kettenmann 2007; Heneka et al. 2014). Activation and response of microglia, a CNS unique myeloid cell population, leads to the production of proinflammatory cytokines of the IL1β family, the release of reactive oxygen species (ROS) and nitric oxide (NO) through the activation of several factors including inducible nitric oxide synthase (iNOS) (Ransohoff and Cadorna 2010). Astrocytes, indeed the most studied neuroglia cells, have several homeostatic functions as buffer CNS potassium, remove toxic glutamate, maintain water balance and modulate synaptic activity. Astrocytes also produce neurotrophins and antiinflammatory cytokines such as IL-10 (Perea et al. 2014). Upon innate immune receptors stimulation (TLRs and NLRs), astrocytes participate in immune reactions producing IL1β, IL6 and lymphocyte/monocytes recall chemokines as CCL2, CXCL2, CXCL10 and CXCL12. In CNS microglia and astrocytes innate immune response plays the alarm for the adaptive response, provided by infiltrating peripheral immune cells. Peripheral immune cells cross the BBB, even when intact, and undergo to different fates, controlled by the BBB itself and the peculiar milieu of the CNS perivascular space (Carson et al. 2006). In physiological conditions neutrophils, eosinophils, monocytes and T lymphocytes infiltrate CNS modestly, on the contrary these cells may be found in brain parenchyma after injures of CNS, as infections and chronic diseases. At the luminal side of BBB leukocytes are used to patrol the barrier and immune surveillance of these cells is directed by adhesion molecules, chemokine and cytokine released by microglia and astrocytes. Cell infiltrates interact with CNS innate immune cells altering a delicate equilibrium: at one side CNS infiltration is critical for defense against infections, on the other inflammation is deleterious for CNS and causes profound tissue damage. Cross talk between CNS innate immune system and infiltrates, T lymphocytes and other peripheral cells, is exemplified in MS and experimental autoimmune encephalomyelitis (EAE, the animal model of MS), focus of the nanomaterials application in the present review.

The molecular mechanisms between neuronal and immune cells are still largely unknown; nonetheless combination of these interactions and nanotechnology has been exploited to improve CNS imaging and drug delivery. In the last years, nanotechnology based drug delivery systems promise to become a good tool to overcame difficulties in crossing the BBB exploiting physiologic mechanisms that normally determine crossing of the barrier or transitory opening of BBB. An alternative, cutting edge strategy to cross this compartmentalized world is to nano-engineer cells of the immune system that normally patrol CNS and enter brain parenchyma.

Nanomaterials and CNS Inflammation: Therapy and Diagnosis Became "Theranostics"

Many biological mechanisms function at the nanoscale. Nanotechnology is a basic science that has permitted the development and characterization of nanostructured materials studied for size and surface properties further developed in CNS nanomedicine as carriers and imaging molecules, with an abundance of different materials with dimensions ranging between 4–10 nm and 100–500 nm (Fig. 1); nanomaterials may be taken up by cells or act depending on the scale as scaffolds for biomolecules present on cell membrane. Consequent to these, bio-nano interactions take place and determine variation in cell adhesion, function and mobility (Miller et al. 2010). Strictly, nanomaterials are defined as functional system composed mostly by particles with a dimension under 100 nm and characterized by properties that are not present at the macroscopic scale. Alternatively to this definition, in nanomedicine the dimension of "nano" is enlarged to 500 nm and even 1000 nm focusing on the ability, due to their dimensions and the still high surface-to-volume ratio, of nanoparticles to alter drug properties (Hofmann-Amtenbrink et al. 2014). Several particles have been reported to cross BBB (reviewed in Shah et al. 2013) and the application field of this technology in brain goes from drug delivery to imaging; to report all the inflammatory/infectious or degenerative CNS diseases where nanomedicine increases the efficacy of treatment is beyond the scope of the present review and is revised elsewhere (Gilmore et al. 2008 3 83–94; Gomes et al. 2014; Wong et al. 2012; Kanwar et al. 2012). Here, we will focus on the special relationship between nanomaterials, nanomedicine and CNS inflammatory responses: neuro-nanomaterials are constantly tested for the risk to provoke inflammation, a too dangerous reaction in CNS tissue, and at the same time are build in order to fight CNS inflammatory diseases. There is a general agreement on the necessity to further investigate nanomaterial biosafety and bioeffects; nonetheless nanomedicine attracts scientific interest particularly concerning the possible application on CNS diseases: today, we have many materials that have been demonstrated to be safe when used at the nanoscale (Jin et al. 2014). Between many characteristics as drug leaking, target specificity and favorable pharmacokinetics a drug nanocarrier must show no toxicity, and above all in CNS to be not toxic goes together with to do not induce inflammatory responses. In this perspective, many studies are performed to determine the surface modification that may increase safety, for example it is known that surface modification with hydrophilic polymer as poly (ethylene glycol) (PEG) increases CNS deliver of nanocarriers improving their stability and lowering inflammatory reactions (Otsuka et al. 2003). Gene and drug delivery is one of the more frequent applications for neuro-nanomaterials, and carbon nanotubes (CNTs) have been exploited to increase poor

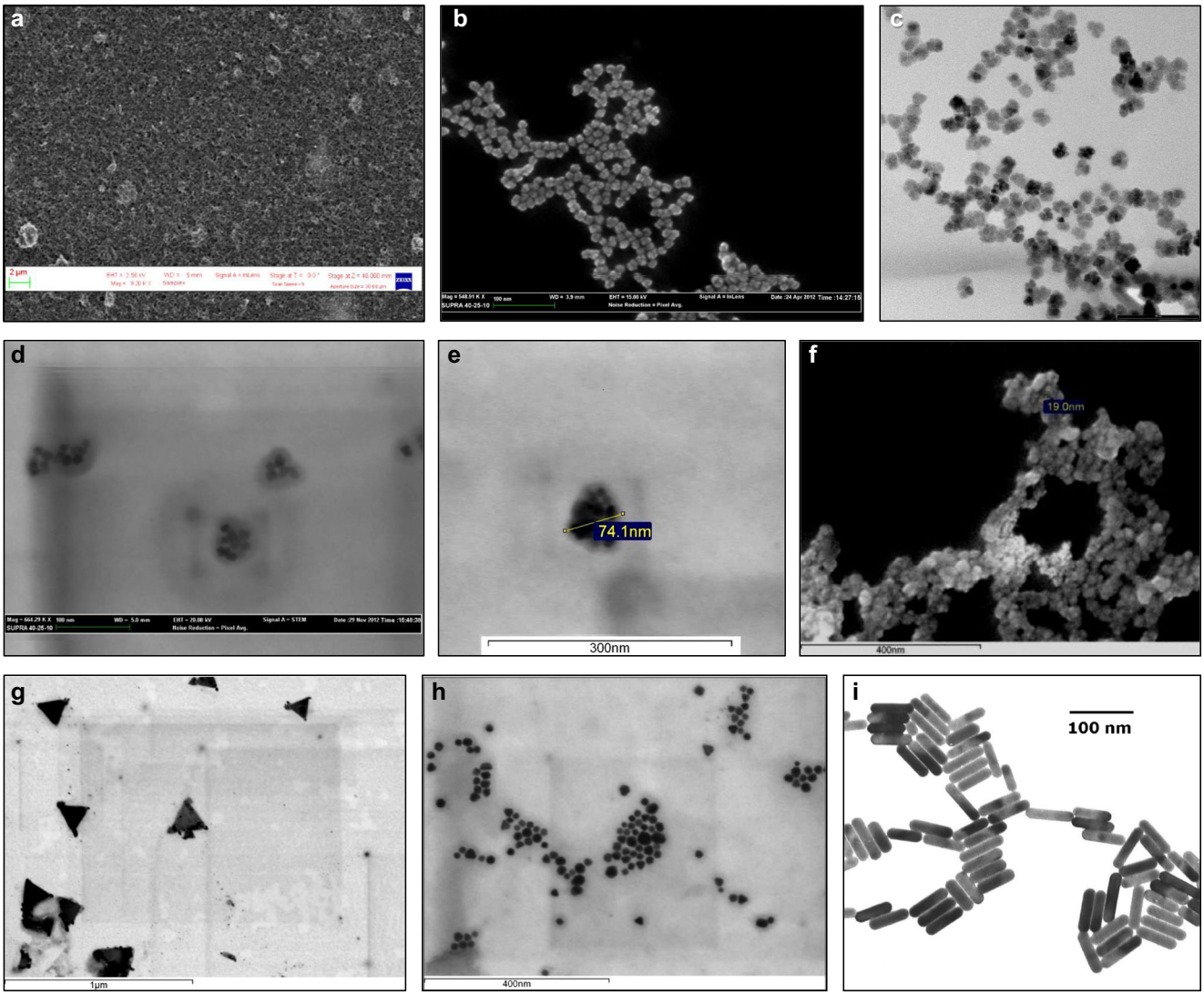

Fig. 1 Examples of nanomaterials. **a** Scanning electron microscopy (SEM) micrograph of a carpet of purified MWCNTs as those used to interface cell growth (Scaini D, Ballerini L, Prato M personal observation). **b** Magnetite nanoparticles Fe_3O_4 picture from Scanning Electron Microscope Field Emission Gun (SEM FEG, Zeiss Supra 40). **c** Magnetite nanoparticles Fe_3O_4 picture from Transmission Electron Microscope (TEM). **d**–**e** Hybrid polymeric/magnetic nanocapsules, core magnetite cluster of nano Fe_3O_4, shell PLGA-PEG Co-polymer picture from SEM FEG (Zeiss Supra 40 STEM mode). **f** Cobalt ferrite nanoparticles $CoFe_2O_4$ picture from SEM FEG (Zeiss Supra 40). **g** Gold triangular shaped nanoparticles picture from SEM FEG (Zeiss Supra 40 STEM mode). **h** Gold rounded shaped nanoparticles picture from SEM FEG (Zeiss Supra 40 STEM mode). **i** Gold nanorod picture from TEM

drug distribution in CNS. In order to avoid the inflammatory response that follows microglia and astrocytes activation due to contact with CNTs, after cortical stereotactic administration, it has been successfully developed a method to functionalize CNT (fCNTs, 200–300 nm) that strongly reduces microglia activation ameliorating the performance of nano carriers (Bardi et al. 2013). Brain inflammatory reaction induced by implants of nanofibrous scaffolds releasing a trophic factor (BDNF mimetic, encapsulated in poly ε caprolactone nano structures) have been investigated in order to evaluate the improvements of neuroblasts migration and neurogenesis together with a reduction in microglia activation and reactive gliosis, both present when implants utilized where scaffolds alone. In this work the authors show that tacking into account possible CNS inflammatory reaction in response to nanomaterial and down modulating it, is crucial for the therapeutic effects of the implants (Fon et al. 2014). Following the same line of research, we recently investigated the ability of human CD14+ derived dendritic cells (DCs, professional antigen presenting cells that take part of CNS infiltrates during inflammation) to sense multi well carbon nanotubes (MWCNT), promising nanostructures in the field or neuroimplants (Cellot et al. 2009). Our data showed that the contact at the nanoscale with MWCNT induced a polarization toward a tolerogenic, less inflammatory DCs (Aldinucci et al. 2013), an encouraging result for future development of

nanotube-neuron devices. Lipid nanoparticles (LNs, 30–50 nm) are biodegradable colloid carriers good for brain targeting (Bondi et al. 2010); it has been shown that PEG modification of LNs reduces particles aggregates dependent microglia activation and consequent induced neuroinflammatory response followed by vascular injury. Once again, determining the nanomaterial risk in inducing neurinflammation permits to individuate promising material for brain drug deliver (Huang et al. 2013). In a recent paper Godinho et al. evaluate the neuroinflammatory effects of non-viral nano vectors (commercial ones compared to modified cationic amphiphilic β-cyclodextrins, CDs) for silencing RNA (siRNA) delivery in the brain. The developed nanocarriers, CDs, compared to commercial ones showed the best balance between efficient silencing and low level of in vitro induced toxic effects on microglia and astrocytes, marker of in vivo nano carrier's ability to trigger neuroinflammation (Godinho et al. 2014). A completely different declination of the effects of nanomaterials at the neuro/immune interface is the reported ability of dendrimers (fluorescent labeled neutral generation-4-polyamidoamine dendrimers, 4 nm) to target in vivo activated astrocytes and microglia following a subarachnoid administration in a rabbit model of cerebral palsy. Future confirmation of this ability, in the absence of targeting ligands, will make this material a feasible way to deliver therapeutics in disorders where astroglia activation and therefore neuroinflammation plays a crucial role, as it happens in MS (Dai et al. 2010). Similarly, in a mouse model of spinal cord injury it has been showed that polymer based nanoparticles (poly-e-caprolactone and poly-ethylene-glycol) loaded with minocycline or poly(−methyl methacrylate) nanoparticles alone selectively target and modulate the associate inflammatory microglia/macrophages reaction present in sub acute progression of secondary injury, maximizing therapeutic efficiency (Papa et al. 2013, 2014). In inflamed CNS, immune cells home to the sites of inflammation and therefore can be exploited as biologically active vehicles. Indeed, this is a frontier aspect of nanomedicine translation in inflamed brain: loading immune cells with nanoformulated drugs to target specific CNS tissue. Klyachko and colleagues loaded a nanozime (catalase nanoformulated with nanoparticles of a copolymer block of PEG; catalase exerts strong antioxidant activity) in alternatively activated mouse bone marrow and human CD14+ derived macrophages. Nanomodified cells were then tested intravenously (i.v.) in an inflamed brain mouse model. Results show that macrophages, loaded with nanozime, were not detected in the brain of healthy animals, whereas were found in inflamed brain. Furthermore, in brain lesion catalase activity was increased, all together these data indicate that macrophages cross BBB and deliver the drug to inflamed tissue (Klyachko et al. 2013).

Neuroimaging with the constant develop and advance of Magnetic Resonance Imaging (MRI) techniques are the primary means for diagnosis in brain inflammatory diseases. MRI can follow in vivo the pathological processes evolving in the brain and is considered an invaluable tool for the diagnosis and follow up of many neurological disorders as well as to study disease pathophysiology. Compared to other imaging techniques, MRI has the clear advantage to be safe and to achieve an exceptional spatial resolution allowing for the visualization of fine anatomical details in the brain. These features make MRI particularly suitable for the application of nanotechnologies in biomedicine and, specifically, to identify pathogenesis, accelerate diagnosis and guide specific therapies for neurological disorders (Smirnov 2009). However, one major limitation of the conventional MRI techniques used in clinical practice, is to lack histopathological specificity. The introduction of novel, nano-sized, molecular contrast agents with MRI offer the adequate sensitivity and specificity to track in real time the pathological processes evolving in the brain. For instance, these contrast agents can be incorporated in specific cells to carry out cellular imaging or bound to specific antibodies to image specific antigens (Vellinga et al. 2008). The most widely used contrast agents in brain nanomedicine are the superparamagnetic and ultrasmall superparamagnetic nanoparticles of iron oxide (respectively SPIO and USPIO) (Weistein et al. 2010). The magnetic signal observed in conventional-clinical MRI, mainly arises from the tissue water protons (Barkhof et al. 2011) and MRI contrast agents can alter the magnetic properties of water protons changing the MRI signal intensity (Maggi et al. 2014). Indeed, the superparamagnetic iron oxide core of SPIO and USPIO behaves like a small local magnet and decreases the longitudinal and transverse relaxation times of water molecules, respectively T1 and T2. This, produces a specific effect in the region where the contrast agent accumulate and a specific increased hyperintense (bright) signal on T1-weighted images and a reduced hypointense (dark) signal on T2 and T2* weighted images (Absinta et al. 2013). In CNS pathology, ferromagnetic nano particles MRI techniques have been applied to image several disorders such as tumors (Neuwelt et al. 2007), stroke (Schefer et al. 2008), Alzheimer disease (Skaat et al. 2013), traumatic brain injury (Sykova and Jendelova 2007), epilepsy (Akhtari et al. 2012) and autoimmune neuroinflammation (McAteer et al. 2007). Ultrasmall superparamagnetic nanoparticles of iron oxide (USPIO) are FDA approved ferromagnetic nanoparticles (10 to 50 nm) that have been frequently used in most contemporary studies on CNS diseases treatment, diagnosis and pathogenesis. These nano particles can be administered i.v. and, compared to Gadolinium (Gd) chelates, they extravasate much slower from the vasculature because of their larger molecular size (up to 50 nm compared to the 1 nm of Gd chelates) (Park et al. 2009). For this reason USPIO based MRI can be used to better asses cerebral perfusion weather Gd

chelates are more suitable contrast agents to estimate BBB permeability (Tofts et al. 1999). Moreover, intravenously injected USPIO nanoparticles are actively taken up by circulating monocytes/macrophages and can be used to target specific inflammatory cells extravasation during brain inflammation (Floris et al. 2004). Other types of cells playing important roles in brain disease pathogenesis and/or therapy, such as lymphocytes (Garden et al. 2006), dendritic cells (de Chickera et al. 2012) and pluripotent stem cells (Ruiz-Cabello et al. 2008), can be labeled in culture with USPIOs providing a unique tool to serially track and quantify the fate of these transplanted cells with MRI (Wu et al. 2008). In CNS tumors, ferromagnetic nanoparticles together with MRI have been used as intravascular contrast agent or cellular imaging contrast agent (Corot et al. 2004). As intravascular contrast agents, USPIO can assess more accurately cerebral perfusion (cerebral blood volume and blood flow) compared to Gd based contrast agents (GBCA) because of their larger molecular size and thus their propensity to remain intravascular at earlier time points (Varallyay et al. 2009). Indeed, cerebral perfusion estimated with MRI and USPIO can give an estimate of the tumor vascularity and neoangiogenesis that are markers of tumoral malignancy and growth (Cha et al. 2002). Moreover, at later time points after intravenous injection of contrast agent, USPIO accumulation within the CNS can be used as a marker for inflammatory cells, being these nano particles actively taken up from circulating monocytes and macrophages that extravasate in the affected tissue. MRI measurements of perfusion parameters with USPIO can also be used to evaluate the efficacy of antiangiogenic therapies (Robinson et al. 2007). In CNS stroke, coupling perfusion and diffusion MRI information has been largely shown to be the gold standard techniques to differentiate infarction versus penumbra. In ischemic CNS lesions, USPIO and MRI were able to visualize areas of inflammatory cells infiltration (USPIO loaded macrophages) (Saleh et al. 2004), a finding that may affect therapeutic strategies in these patients. Macrophage infiltration in atherosclerotic carotid plaques can also be highlighted using USPIO based MRI, and could be potentially used as a screening tool to reduce the incidence of stroke in asymptomatic plaques (Tang et al. 2006).

Multiple Sclerosis

MS is a chronic, inflammatory demyelinating disease of the CNS characterized by clinical heterogeneity with a primary progressive form (PP) in 20 % of patients and a relapsing remitting (RR) evolving into secondary progressive (SP) form in the 65 % of cases (Lassmann et al. 2012). MS etiology is unknown, but epidemiological studies indicate that MS, a multifactorial, complex trait disease, arises by genetically determined susceptibility and environmental factors. MS complicate pathology involves many different actors: effector immune cells, cytokines, chemokines, growth factors and other molecules as adhesion molecules contribute to the disease onset and progression together with free radicals, proteases and amines. Plaques that are distributed in subcortical and periventricular white matter, optic nerve, brain stem and spinal cord characterize RR MS pathology. Typical focal lesions contain perivascular infiltrates where mainly CD8+ and CD4+ T lymphocytes, monocytes, dendritic cells (DCs) and rare B lymphocytes and plasma cells are found together with macrophages containing myelin debris (Nylander and Hafler 2012). For these features, MS is a prototype of inflammatory autoimmune disorder, where CNS resident immune cells and infiltrating inflammatory ones encounter and react with neurons, axons and neuroglia cells. This scenario change in progressive stage of the disease, where extensive axonal damage is associated with demyelination and gradual lesion expansion takes place with abnormal inflammation of normal appearing white matter. Irreversible axonal loss is a neuropathological hallmark of progressive disease and the presence of a neurodegenerative component in this phase is in keeping with clinical evidences that immunomodulatory or immunosuppressant drugs, typically acting during RR MS, are ineffective. In the last years, immune mechanisms associated with MS have been described and as consequences many therapies have been developed for the early, relapsing form of the disease. On the contrary, therapies developed to protect and repair injured axons and neurons or able to induce remyelination and cure progressive form of the disease still lack (Hauser et al. 2013).

Many of the MS therapies have been developed in the animal model for MS: experimental autoimmune encephalomyelitis (EAE), a model where proinflammatory immune response against myelin is the principal mechanism driving pathogenesis. Indeed, EAE is the best known and characterized model for MS. The disease is induced by active immunization of recipient animals with myelin antigens together with an adjuvant or by passive transfer of CNS antigen specific T cell line. Actively induced EAE consists of an induction phase and an effector phase. The induction phase of the disease involves the priming of myelin epitope–specific CD4 + T cells following immunization with myelin proteins or peptides in complete Freund's adjuvant (CFA). The effector phase consists of multiple stages: migration of activated myelin-specific T cells to the CNS, which involves extravasation of the T cells across the tight endothelial junctions comprising the BBB; production of chemokines and cytokines by the myelin-specific T cells, which induce peripheral mononuclear phagocytes influx into CNS parenchyma; activation of peripheral monocytes/macrophages and CNS-resident microglia cells by T cell–derived cytokines; and demyelination of CNS axonal tracts by the phagotycic activity of activated mononuclear

cells and by the inflammatory and cytotoxic effects of cytokines (e.g. IL-17, IFN-γ, TNF-β, TNF-α, and NO) released from activated CD4+ T cells and monocytes. This causes an autoimmune reaction against myelin in the CNS of the immunized animals. EAE can be induced in a large variety of species (mice, rats, guinea pigs, monkeys) among which mice are the most commonly used. Different types of EAE depend on antigens, and the reduction in complexity of the antigenic material from crude brain tissue and protein extracts through various central myelin proteins such as myelin basic glycoprotein (MBP), myelin oligodendrocytes glycoprotein (MOG), proteo lipid protein (PLP), and small encephalytogenic peptide. The clinical features of disease vary among different species and among different models (based on the type of myelin antigen used to induce the disease) ranging from an acute monophasic disease to a relapsing-remitting one closer to human MS. What associates MS and EAE is the histopathology of the acute lesion, where inflammatory infiltrates, mostly T-cells and macrophages, are cuffed around post capillary venules (Baxter 2007). In both diseases, demyelination is inevitably linked to the presence of activated macrophages that remove myelin by phagocytosis (Prineas and Parratt 2012). Due to the strong immunization protocol that causes tolerance rupture in EAE, it is possible that the first phase of the immune response—that is, why and how the immune system starts to react against the CNS—differs between the two diseases. Nonetheless, there is a close histopathological similarity between MS and EAE suggesting that the consequent effector phase of the immune response may be very similar (t' Hart and Massacesi 2009). For this reason, EAE is considered an invaluable model to study the pathophysiology of MS. To date, three important clinical therapeutics commonly used to treat MS patients have been developed in the EAE model: glatiramer acetate, mitoxantrone and natalizumab (Yednock et al. 1992). However, a large number of other potential therapies effective in mice EAE failed to have clinical efficacy in human MS (Sriram and Steiner 2005; Steinmann and Zamvil 2006). This is likely to be due to the large evolutionary distance between rodents and humans. Therefore the development of EAE models in species with a closer evolutionary relationship to human would help to bridge the gap between mice EAE and human MS. Non human primates are genetically, immunologically, and microbiologically closer to humans, and for this reason EAE has been developed in old and new world monkeys. EAE in macaques (old world monkeys) is characterized by hyperacute destructive CNS disease causing the death of the animal within a few days after onset (t Hart et al. 2000). Over the course of the years, EAE in the marmoset has been shown to be clinically, immunologically and pathologically closer to human MS than EAE in other species and may be an attractive model for preclinical studies of new therapeutics. Furthermore, the relatively small size of these primates and the higher brain white-gray matter ratio compared to rodents (considering that WM lesions are an important feature of MS pathology) makes them very suitable for MRI studies on disease pathogenesis.

Nanomaterials and Multiple Sclerosis: Therapy

Nanomaterials, in designing MS therapy, have a wide field of actions: they help to obtain efficient systems for CNS drug/gene delivery, tolerance-inducing nanocarriers, tolerance inducing vaccine and platforms for screening regenerative therapeutics. Most of the studies presented here are preclinical studies mainly conduct on EAE models (Table 1), this is due to the still limited translation of nanotechnology into clinic: long term toxicity and homogeneous nano preparations are the main difficulties that need to be overcome to permit expansion of this new therapeutic approach.

Liposome preparations have been widely used in EAE treatment following two main therapeutic strategies: drug delivery and tolerance induction (Strejan and St Louis 1990; Cavaletti et al. 2009; Schmidt et al. 2003). More recently, nano-sterically stabilized liposome (nSSL, first nanoliposome FDA approved for anticancer therapy) loaded with glucocorticoids (methylprednisolone hemisuccinate sodium salt) were demonstrated of therapeutic efficacy in the PLP-induced mouse EAE and this treatment was superior in term of efficacy compared to free drug. In animals treated with nSSL, recovery from acute disease was faster even compared with clinically used MS drugs: Betaferon and Copaxone (Avnir et al. 2011). In another work, Tempamine loaded nSSL were i.v. injected in the SJL PLP and C57/Bl6 MOG induced EAE to counteract impaired cell redox balance and autoimmune reaction, components of MS and EAE pathogenesis. Thanks to nSSL ability to efficiently cross BBB, nano liposome reached inflamed brain and ameliorate acute and chronic EAE in terms of clinical score and number of infiltrates (Kizelsztein et al. 2009). Cerium^{iv} oxide nanoparticles, stabilized with citrate/EDTA showed, after i.v administration, to act as antioxidant in CNS reducing reactive oxygen species level in a C57/Bl6 MOG EAE mice. This treatment alleviates clinical symptoms and motor deficit, mitigating CNS damage derived from free radical accumulation (Heckman et al. 2013). Nanotechnology offers a new method to re-establish tolerance by expanding antigen specific T regulatory cells and consequent treatment of autoimmune diseases including MS. A recent study shows the potent immune regulatory effects of DNA nanoparticles prepared by mixing polymer a poly-ethylenenimine polymer and cargo DNA, and administered i.v. at various time in EAE MOG mice. This treatment reduces disease severity and delays disease onset by regulating IDO dependent T cell response (Lemos et al. 2014).

Nanomaterials are frequently utilized in vaccine-like therapeutic tools. In SJL/J PLP relapsing EAE mice, intravenous infusion of polystyrene or biodegradable

Table 1 Nanomaterials and MS: therapy and imaging

Nanomaterial	Functionalization	Ligands	Application							References
			Drug/ gene delivery	Tolerance induction	Antioxidant	Cell targeting	Apoptosis induction	Screening regenerative therapeutics	MRI	
Liposomes	Positive charged; nano-sterically stabilized liposome (nSSL)	Glucocorticoids; tempamine	×	×	×	×				Strejan et al 1990; Schmidt et al 2003; Cavaletti et al 2009; Avnir et al 2011; Kizelsztein et al 2009
Polyethylenenimine		cargo DNA	×			×				Lemos et al 2014
Poly(lactic-co-glycolic) (PLGA) nanoparti-cles	Poly(ethylene glycol) (PEG); poly(ethylene-co-maleic acid) (PEMA); negatively charged immune modifying particles (IMP)	Myelin antigens; peptide Ac-PLP-BPI.NH2-2 (binding MHCII and ICAM1); MOG_{35-55}+ recombinant IL10		×		×	×			Getts et al. 2012; Getts et al. 2011; Büyüktimkin et al. 2012; Cappellano et al. 2014; Hunter et al 2014; Getts et al. 2014
Polystyrene	Negatevely charged IMP					×	×			Getts et al. 2014
Microdiamonds	Negatevely charged IMP					×	×			Getts et al. 2014
Gold nanoparticles	PEG	MOG_{35-55}+2-(1′H-indole-3′-carbonyl)-thyazole-4-carboxylic acid methyl ester (ITE)		×						Yeste et al. 2012
DNA-peptide nanoparticles		MOG_{35-55}+BTLA		×		×				Yuan et al. 2014
Cerium oxide nanoparticles	Citrate/EDTA				×	×				Heckman et al 2013
Silica nanofibers	Micropillar array engineered in a conical shape (BIMA)								×	Mei et al. 2014
Superparamagnetic and ultrasmall superparamagnetic nanoparticles of iron oxide (SPIO, USPIO)		Anti-CD3 mAb; anti-B220 mAb; combined use of Gadolinium based contrast agents				×			×	Anderson et al 2004; Baeten et al 2008; Baeten et al 2010; Oude Engberink et al. 2008; Vellinga et al 2008; Luchetti et al 2011; Floris et al 2004; McAteer et al. 2007; Garden et al 2006; Kap et al 2011

poly(lactic-co-glycolic) (PLGA) microparticles (500 nm) bearing encephalytogenic peptides induce antigen specific T cell anergy and the consequent induction of tolerance prevents EAE. These effects are mediated by particles uptake in spleen marginal zone trafficking macrophages expressing a specific scavenger molecule. In this setting, the particles act as efficient substitutes for apoptotic cells coupled with antigenic peptides, previously described by the same authors as a good treatment in preclinical models of MS (Getts et al. 2011, 2012). Targeted tolerance induction by i.v. infusion of poly(lactic-co-glycolic acid) PLG nanoparticles (500 nm) modified with the surfactant poly(-ethylene-co-maleic acid) PEMA and coupled with myelin antigens, has been showed highly efficient in antigen specific T cell tolerance induction and in treatment of relapse EAE mouse model of MS. When myelin antigen coupled PLG nanoparticles were administered at onset or at peak of acute EAE they ameliorate ongoing disease. PLG/PEMA modified nanoparticles are a safe platform system inducing antigen specific tolerance by combining anergy and Treg expansion, probably this is due to the favorable combination of shape, surface and size (Hunter et al. 2014). PLP SJL/J EAE mice have been treated subcutaneously (s.c.) with a vaccine-like therapy with a complex of alginate chitosan PLGA nanoparticles forming a colloidal gel added by the peptide Ac-PLP-BPI.NH2-2, designed to bind MHCII and adhesion molecule (ICAM1) simultaneously. The treatment was effective in long term suppression of Th17 cell, probably blocking the formation of the immune synapse, responsible of encephalytogenic T cell activation; furthermore the treatment, thanks to this nano-formulation, was efficient with one time injection (Büyüktimkin et al. 2012). Recently s.c. in vivo inverse vaccination procedure that inhibits autoimmune response in C57/Bl6 EAE mice by antigen specific tolerogenic immunization with $MOG_{35–55}$ loaded PLGA nanoparticles together with recombinant IL10 (rIL10), has been demonstrated effective in EAE following two different vaccination protocols: prophylactic and therapeutic. The solid biodegradable particles utilized here guarantee the sustained release of antigen and adjuvant with a significant protective effect on EAE (Cappellano et al. 2014). Boosting Foxp3+ expression and consequent Treg expansion may induce tolerance and EAE protection. RNS60 modified saline, a nano-bubbles composition with diameter between 26 and 33 nm, restores Tregs response in MBP-primed T lymohocytes with shift of CD4 + Th1 into Th2 during autoimmune reaction. This is due to RNS60 regulation of NO production, that results inhibited by the treatment and consisting with in vitro findings, RNS60 treatment (i.p. 300 μl/mouse /d) determines amelioration of adoptive transfer SJL RR EAE by Tregs induction, providing a novel mechanism of immune-modulation (Mondal et al. 2012).

In our introduction we underlined the necessity to develop materials that avoid immune mediated clearance; nonetheless, as it has been showed, this nanoparticles surface feature may be translated into therapy. Eliminating inflammatory macrophages represents an alternative strategy to reduce tissue injury in MS, EAE and other autoimmune diseases. In this perspective, Getts et al. showed that infusion of negatively charged immune-modifying particles (IMPs,) derived from polystyrene, microdiamonds or biodegradable PLGA (PLGA-IMP) induces apoptosis of spleen trafficking macrophages reducing their accumulation at inflammation foci with consequent amelioration of disease symptoms in EAE mice and other immune mediated disease animal models, where inflammatory monocytes have a crucial role in mediating tissue damage mechanisms (Getts et al. 2014). Shifting nanomaterials action from monocytes to DCs, parenteral administration of PEG stabilized gold nanoparticles carrying $MOG_{35–55}$ peptide and ITE (an 2-(1′H-indole-3′-carbonyl)-thyazole-4-carboxylic acid methyl ester that induces through different ligands DCs to promote regulatory FOXP3+ T cells) suppresses the development of EAE re-establishing tolerance to encephalytogenic myelin antigen (Yeste et al. 2012). In another work the double nature of DCs, immunogenic or tolerogenic, has been exploited and nano-engineered DCs have been transferred into MOG EAE mice in order to induce specific CD4+ T lymphocytes tolerance and treat EAE. DCs were modified by mean of transduction of nanoparticles containing BTLA (membrane glycoprotein of the CD28 super family, a T cells, B cells receptor attenuator) and a MOG peptide, in vivo the modified cells significantly enhanced Foxp3+ CD4+ T regulatory cells and suppress CD4+ T cell response to MOG, in EAE spinal cord number of infiltrates was reduced and disease mostly suppressed (Yuan et al. 2014). Remyelination is a crucial issue in MS therapy and is rather difficult to evaluate possible drugs improvement of oligodendrocytes ability to regenerate and reestablish normal myelin sheets after autoimmune damage. The myelination process involves direct interaction between oligodendrocytes and neurons and this interaction is hard to be followed in vivo or modeled in vitro. Nanomaterials and nanotechnology have recently permitted the development of screening platforms in order to evaluate the potential of regenerative drugs in MS. Recently, freestanding silica nanofibers organized as micropillar array engineered in a conical shape (BIMA) were utilized as pseudo axonal substrate, sufficient for screening oligodendrocytes myelination in a cell autonomous manner. These platforms permitted to correctly evaluate possible therapeutics able to induce remyelination, with a deep insight into mechanisms and pathways necessary for oligodendrocytes development and myelination (Mei et al. 2014).

Nanomaterials and Multiple Sclerosis: Imaging

Lesions in MS are demyelinated, can occur both in white and gray matter, and tend to develop around parenchyma veins (Adams and Kubik 1952). A classic feature of early MS lesions is the presence of a cellular inflammatory cuff, mostly lymphocytes and dendritic cells, around the central vein (Prineas and Parratt 2012). These early MS lesions, commonly known as acute lesions, are associated with profound leakage of the BBB allowing inflammatory cells and factors to enter the lesion parenchyma (Katz et al. 1993). In the parenchyma surrounding the central inflamed blood vessel, myelin is usually damaged and picked up from large activated macrophages that are, for the most part, blood derived monocyte-macrophages expressing marker of early-stage activation. Over the course of days-weeks the inflammatory process tends to spread out from the initial area immediately surrounding the central vein in the near by parenchyma (Gaitan et al. 2011).

In basic MS-MRI research, the great advantage of studying these pathological processes in animal models is to allow the comparison between in vivo MRI images and ex vivo histopathology (Fig. 2), a perspective rarely obtainable in human MS MRI studies.

EAE is the best known and characterized model for MS and the clinical features of disease vary among different species and among different models; what really associates MS and EAE is the histopathology of the acute lesion, where inflammatory infiltrates, mostly T-cells and macrophages, are cuffed around post capillary venules (Baxter 2007). As remembered before, EAE is considered an invaluable model to study the pathophysiology of MS. One obvious advantage to study the animal model over the human disease is the possibility to easily access to premorbid images as well as to compare in vivo MRI images with ex-vivo histopathology. USPIO based MRI applied to the EAE model, offers the unique opportunity to study specific processes that occurs early during disease pathogenesis as well as to follow the fate of specific inflammatory cell subtypes, mostly T cells and/or macrophages, during the process of WM inflammatory lesion formation (Anderson et al. 2004; Baeten et al. 2008, 2010).

Both the activation of cerebral blood vessel, consisting in the expression of adhesion molecules on endothelial cells surface, and the increased of cerebral blood vessel permeability are changes that occur early during the development of an inflammatory demyelinated lesion (Reboldi et al. 2009). MRI traceable ferromagnetic nano particles combined with antibodies specific for endothelial adhesion molecules or membrane T lymphocytes molecules (CD3) have thus been used to in vivo detect activated cerebral blood vessels during brain inflammation or migratory pathway of infiltrating T cells (Luchetti et al. 2011), a finding that may accelerate diagnosis at time when pathology is otherwise undetectable. The combined

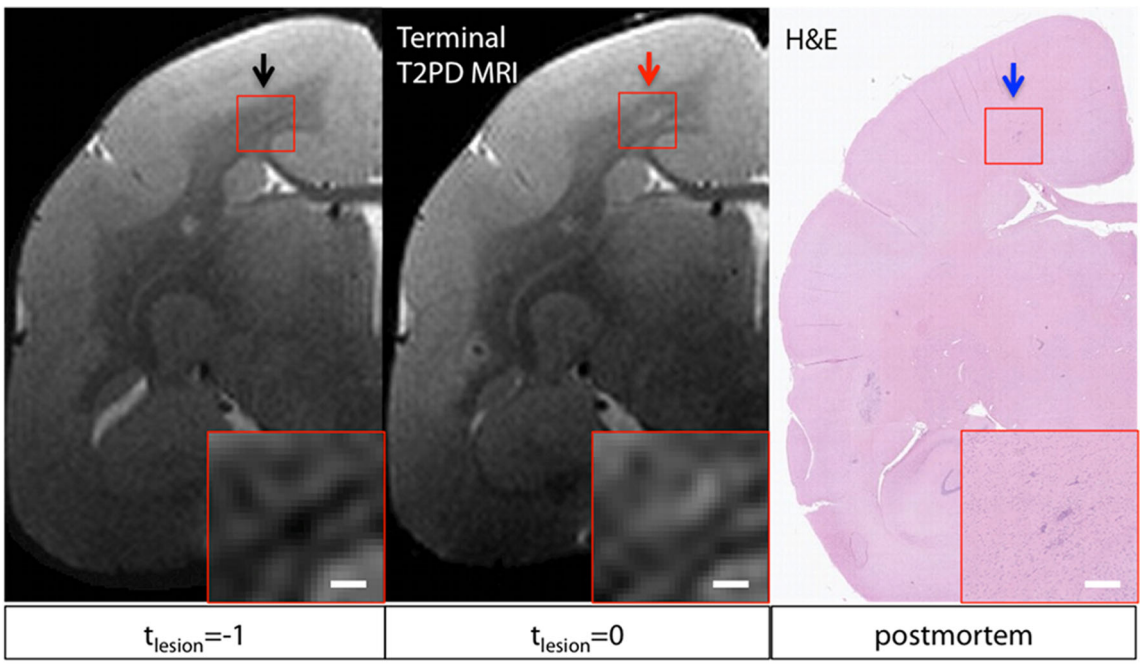

Fig. 2 Arithmetic average between PD and T2 weighted magnetic resonance imaging (T2PD MRI) and histopathological features of an acute lesion (less than 1 week old) from a Common Marmoset immunized with human white matter homogenate as previously described (Gaitan et al. Mult Scler 2013). **a** The lesion was clearly visible on the terminal MRI (t_{lesion}=0) but not present on the MRI performed 1 week earlier (t_{lesion}=−1). The same lesion was found on the matched hematoxylin and eosin (H&E) section (*blue arrow*)

use of Gd based contrast agents and USPIO with MRI can help to identify the sequence of events that set the stage for tissue damage during brain inflammation in both EAE (Floris et al. 2004) and MS (McAteer et al. 2007). Indeed, an increased in BBB permeability as detected from Gd leakage preceded USPIO labeled macrophage infiltration during the development of inflammatory lesion in EAE, a finding consistent with recent data in newly forming WM inflammatory lesion in EAE (Maggi et al. 2014). Specifically, USPIO would be incorporated by monocyte/macrophages circulating in the peripheral blood stream. USPIO particles would be then vehicled in the inflamed CNS lesions during the process of macrophage extravasations, although other mechanisms contributing to USPIO MRI enhancement in the brain (such as BBB leakage) cannot be excluded (Oude Engberink et al. 2008). The pathological analysis of inflammatory EAE lesions showed how USPIO were present within early activated, newly infiltrating macrophages but not in perivascular macrophages. Notably, both in MS and EAE, USPIO pattern of enhancement significantly differs from the pattern of gadolinium enhancement in time (Vellinga et al. 2008).

Tracking myelin reactive, encephalitogenic T cells with USPIO based MRI techniques offers the opportunity to follow the fate of these cells during the development of EAE (Herz et al. 2011), possibly giving new pathophysiological insights about the process of WM lesion formation. Myelin reactive T cells can be labeled in vitro with ferromagnetic nano particles and then transferred into the animal. The injection of these cells at different stages of disease progression, i.e. in naïve or in already actively immunized animals, was found to correlate with the distribution of the inflammatory T cells within the inflamed brain and spinal cord (Garden et al. 2006). This kind of approach, possibly applied to EAE models in species with a closer evolutionary relationship to human (Kap et al. 2011), could help to unravel the precise role of specific inflammatory cell subtypes during the process of inflammatory lesion formation, possibly opening the door to the development of new therapeutics aimed to prevent the developments and progression of neurological disability.

Furthermore, such cellular imaging techniques may allow for a more accurate MRI characterization of disease activity in later stage of multiple sclerosis when a decreased number of gadolinium enhancing lesions is seen (McFarland 1998), despite increasing neurologic deficit. Indeed, in older lesions and particularly those found during the progressive stage of MS, inflammation is subtle if present (Soon et al. 2007; Ge et al. 2005) and is not usually detected from conventional Gd based MRI techniques. The possibility to evaluate the presence of low-grade inflammation and inflammatory cells infiltration in these lesions with USPIO MRI, could affect therapeutic strategies and therapy development.

Conclusion

Recent developments of nanotechnology and nanomaterials have gave a huge contribute in CNS diagnosis and therapy. In MS, where local and infiltrated immune cells encounter neuronal cells and myelin producing oligodendroglia arising a complex pathological mechanism hidden by the BBB and by a compartmentalized inflamed environment, nanomaterials able to target lesion and to deliver drugs in a more efficient way, represent a solid therapeutic approach for the near future. Nanotechnology centered therapeutic approaches are of various nature. Here we have described the improvement that particles that cross BBB and reach inflamed brain may bring as drug deliver and we indicate all the different applications of nanoparticles in tolerance induction, for example tolerance is efficiently induced by particles carrying encephalytogenic peptides and rIL10. Finally we pointed out the discovery of particles that, for specific physicochemical properties act as antioxidant agents and open a new era in the treatment of CNS injured tissue. Between the multiple strategies reported, the cutting edge idea is that nanomaterials may engineer cells, as the case of gold particles on DCs that shift cells toward tolerogenic functions or nanoparticles that induce apoptosis on peripheral macrophages; this aspect is a stimulus and a warning to the future utilization of nanomaterials: cells sense material at the nanoscale and react. During the last years many progresses have been made in imaging an inflamed brain and in this field USPIO are the nanoparticles that better meet the needs of MRI MS diagnosis: they are new diagnostic agents, they enhance lesions and early processes that Gd is not able to identify. Furthermore, USPIO by being internalized in infiltrating cells may permit to follow the fate of specific cells subtypes or track encephalytogenic T cells and all these features will give new MS pathophysiological insights.

Acknowledgments We are specially grateful to Dennis Scaini, Laura Ballerini and Maurizio Prato for providing us the SEM micrograph of purified MWCNTs original imagine and for helpful comments and manuscript critical revision. We are grateful to Sheila Macri, Emily Leibovitch, Maria Gaitan, Afonso Silva, Susan Westmoreland, Steve Jacobson and Daniel Reich for MRI/histology images in marmoset EAE.

Conflict of interest The authors declare that they have no conflict of interest

References

Abbott NJ, Patabendige AA, Dolman DE et al (2010) Structure and function of the blood brain barrier. Neurobiol Dis 37:13–25

Absinta M, Sati P, Gaitan MI et al (2013) Seven-tesla phase imaging of acute multiple sclerosis lesions: a new window into the inflammatory process. Ann Neurol 74(5):669–678

Adams RD, Kubik CS (1952) The morbid anatomy of demyelinative disease. Am J Med 12(5):510–546

Akhtari M, Bragin A, Moats R et al (2012) Imaging brain neuronal activity using functionalized magneto particles and MRI. Brain Topogr 25(4):374–388

Aldinucci A, Turco A, Biagioli T et al (2013) Carbon nanotube scaffolds instruct human dendritic cells: modulating immune responses by contacts at the nanoscale. Nano Lett 13(12):6098–6105

Anderson SA, Shukaliak-Quandt J, Jordn EK et al (2004) Magnetic resonance imaging of labeled T-cells in a mouse model of mutliple sclerosis. Ann Neurol 55(5):654–659

Avnir Y, Turjeman K, Tulchinsky D et al (2011) Fabrication principles and their contribution to the superior in vivo therapeutic efficacy of nanoliposomes remote loaded with glucocorticoids. PLoS One 6(10):e25721

Baeten K, Hendriks JJ, Hellings N et al (2008) Visualization of the kinetics of macrophage infiltration during experimental autoimmune encephalomyelitis by magnetic resonance imaging. J Neuroimmunol 195(1–2):1–6

Baeten K, Adriaensens P, Hendriks J et al (2010) Tracking of myelin reactive RT cells in EAE animals using small particles of iron oxide and MRI. NMR Biomed 23(6):601–619

Bardi G, Nunes A, Gherardini L et al (2013) Functionalized carbon nanotubes in the brain: cellular internalization and neuroinflammatory response. PLoS One 8(11):e80964

Barkhof F, Simon JH, Fazekas F et al (2011) MRI monitoring of immunomodulation in relapse-onset multiple sclerosis trials. Nat Rev Neurol 8(1):13–21

Baxter AG (2007) The origin and application of experimental autoimmune encephalomyelitis. Nat Rev Immunol 7(11):904–912

Bondi ML, Craparo EF, Giammona G et al (2010) Brain-targeted solid lipid nanoparticles containing riluzole: preparation, characterization and biodistribution. Nanomedicine 5:25–32

Büyüktimkin B, Wang Q, Kiptoo P et al (2012) Vaccine-like controlled-release delivery of an immunomodulating peptide to treat experimental autoimmune encephalomyelitis. Mol Pharm 9:979–985

Cappellano G, Woldetsadik AD, Orilieri E (2014) Subcutaneous inverse vaccination with PLGA particles loaded with a MOG peptide and IL-10 decreases the severity of experimental autoimmune encephalomyelitis. Vaccine 32:5681–5689

Carson MJ, Doose JM, Melchior B et al (2006) CNS immune privilege: hiding in plain sight. Immunol Rev 213:48–65

Cavaletti G, Cassetti A, Canta A (2009) Cationic liposomes target sites of acute neuroinflammation in experimental autoimmune encephalomyelitis. Mol Pharm 6(4):1050–1058

Cellot G, Cilia E, Cipollone S et al (2009) Carbon nanotubes might improve neuronal performance by favoring electrical shortcuts. Nat Nanotechnol 4:126–133

Cha S, Knopp EA, Johnson G, et al (2002) Intracranial mass lesions: dynamic contrast-enhanced susceptibility-weighted echo-planar perfusion MR imaging. Radiol 223(1):11–29

Corot C, Petry KG, Trivedi R et al (2004) Macrophage imaging in central nervous system and in carotid atherosclerotic plaque using ultrasmall superparamagnetic iron oxide in magnetic resonance imaging. Investig Radiol 39(10):619–625

Dai H, Navath RS, Balakrishnan B et al (2010) Intrinsic targeting of inflammatory cells in the brain by polyamidoamine dendrimers upon subaracnoid administration. Nanomedicine 5(9):1317–1329

de Chickera S, Willert C, Mallet C et al (2012) Cellular MRI as suitable, sensitive non-invasive modality for correlating in vivo migratory efficiencies of different dendritic cell populations with subsequent biological outcomes. Int Immunol 24(1):29–41

Floris S, Blezer EL, Schreibelt G et al (2004) Blood–brain barrier permeability and monocyte infiltration in experimental allergic encephalomyelitis: a quantitative MRI study. Brain 127(3):616–627

Fon D, Zhou K, Ercole F et al (2014) Nanofibrous scaffolds releasing a small molecule BDNF-mimetic for the re-direction of endogenous neuroblast migration in the brain. Biomaterials 35:2692–2712

Gaitan MI, Shea CD, Evangelou IE et al (2011) Evolution of the blood–brain barrier in newly forming multiple sclerosis lesions. Ann Neurol 70(1):22–29

Gaitan MI, Sati P, Inati SJ et al (2013) Initial investigation of the blood–brain barrier in MS lesions at 7 tesla. Mult Scler 19(8):1068–1073

Garden OA, Reynolds PR, Yates J et al (2006) A rapid method for labelling CD4 + T cells with ultrasmall paramagnetic iron oxide nanoparticles for magnetic resonance imaging that preserves proliferative, regulatory, and migratory behavior in vitro. J Immunol Methods 314(1–2):123–133

Ge Y, Law M, Johnson G et al (2005) Dynamic susceptibility contrast perfusion MR imaging of multiple sclerosis lesions: characterizing hemodynamic impairment and inflammatory activity. Am J Neuroradiol 26(6):1539–1547

Getts DR, Turley DM, Smith CE et al (2011) Tolerance induced by apoptotic antigen-coupled leukocytes is induced by PD-L1+ and IL-10 producing splenic macrophages and maintained by T regulatory cells. J Immunol 187:2405–2417

Getts DR, Martin AJ, McCarthy DP et al (2012) Microparticles bearing encephalytogenic peptides induce T-cell tolerance and ameliorate experimental autoimmune encephalomyelitis. Nat Biotechnol 30(12):1217–1224

Getts DR, Terry RL, Getts MT et al (2014) Therapeutic inflammatory monocyte modulation using immune-modifying microparticles. Sci Transl Med 6:219ra7

Gilmore JL, Yi X, Quan L et al (2008) Novel nanomaterials for clinical neuroscience. J NeuroImmune Pharm 3:83–94

Godinho BM, McCarthy DJ, Torres-Fuentes C et al (2014) Differential nanotoxicological and neuroinflammatory liabilities of non-viral vectors for RNA interference in the central nervous system. Biomaterials 35:489–499

Gomes MJ, Neves JD, Sarmento B (2014) Nanoparticle-based drug delivery to improve the efficacy of antiretroviral therapy in the central nervous system. Int J Nanomedicine 9:1757–1769

Hanisch UK, Kettenmann H (2007) Microglia. active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10(11):1387–1394

Hauser SL, Chan JR, Oksenberg JR (2013) Multiple Sclerosis: prospects and promise. Ann Neurol 74:317–327

Heckman KL, DeCoteau W, Estevez A et al (2013) Custom cerium oxide nanoparticles protect against a free radical mediated autoimmune degenerative disease in the brain. ACS Nano 7(12):10582–10596

Heneka MT, Kummer MP, Latz E (2014) Innate immune activation in neurodegenerative disease. Nat Rev Immunol 14(7):463–477

Herz J, Paterka M, Niesner RA et al (2011) In vivo imaging of lymphocytes in the CNS reveals different behavior of naive T cells in health and disease. J Neuroinflammation 8:131

Hofmann-Amtenbrink M, Hofmann H, Hool A et al (2014) Nanotechnology in medicine: European research and its implications. Swiss Med Wkly 144:w14044

Huang JY, Lu YM, Wang H et al (2013) The effect of lipid nanoparticle PEGylation on neuroinflammatory response in mouse brain. Biomaterials 34:7960–7970

Hunter Z, McCarthy DP, Yap WT et al (2014) A biodegradable nanoparticle platform for the induction of antigen-specific immune tolerance for treatment of autoimmune disease. ACS Nano 8(3):2148–2160

Jin S, Li S, Wang C et al (2014) Biosafe nanoscale pharmaceutical adjuvant materials. J Biomed Nanotechnol 10(9):2393–2419

Kanwar JR, Sun X, Punj V et al (2012) Nanoparticles in the treatment and diagnosis of neurological disorders: untamed dragon with fire power to heal. Nanomedicine: nanotechnology. Biol Med 8:399–414

Kap YS, Laman JD, 't Hart BA (2011) Effects of early IL17A neutralization on disease induction in a primate model of experimental autoimmune encephalomyelitis. J NeuroImmune Pharm 5(2):220–230

Katz D, Taubenberger JK, Cannella B et al (1993) Correlation between magnetic resonance imaging findings and lesion development in chronic, active multiple sclerosis. Ann Neurol 34(5):661–669

Kizelsztein P, Ovadia H, Garbuzenko O et al (2009) Pegylated nanoliposomes remote-loaded with the antioxidant tempamine ameliorate experimental autoimmune encephalomyelitis. J NeuroImmune 213:20–25

Klyachko NL, Haney MJ, Zhao Y (2013) Macrophages offer a paradigm switch for CNS delivery of therapeutic proteins. Nanomedicine 9(9): 1403–1422

Lassmann H, van Horssen J, Mahad D (2012) Progressive multiple sclerosis. Nat Rev Neurol 8:647–656

Lemos H, Huang L, Chandler PR et al (2014) Activating of the STING adaptor attenuates experimental autoimmune encephalomyelitis. J Immunol 192:5571–5578

Luchetti A, Milani D, Ruffini F et al (2011) Monoclonal antibodies conjugated with superparamagnetic iron oxide particles allow magnetic resonance imaging detection of lymphocytes in the mouse. Mol Imaging 2011:1–12

Maggi P, Macri SM, Gaitan MI et al (2014) The formation of inflammatory demyelinated lesions in cerebral white matter. Ann Neurol 76(4):594–608

McAteer MA, Sibson NR, von Zur MC et al (2007) In vivo magnetic resonance imaging of acute brain inflammation using microparticles of iron oxide. Nat Med 13(10):1253–1258

McFarland HF (1998) The lesion in multiple sclerosis: clinical, pathological, and magnetic resonance imaging considerations. J Neurol Neurosurg Psychiatry 64(Suppl 1):S26–S30

Mei F, Fancy SP, Shen YA et al (2014) Micropillar arrays as high-throughput screening platform for therapeutics in multiple sclerosis. Nat Med 20(8):954–961

Miller IS, Lynch I, Dowling D et al (2010) Surface induced cell signaling events control actin rearrangements an motility. J Biomed Mater Res A 93:493–504

Mondal S, Martinson JA, Ghosh S et al (2012) Protection of Tregs, suppression of Th1 and Th17 cells, and amelioration of experimental allergic encephalomyelitis by physically-modified saline. PLoS ONE 7(12):e51869. doi:10.13171/journal.pone.0051869

Neuwelt EA et al (2007) The potential of ferumoxytol nanoparticle magnetic resonance imaging, perfusion and angiography in central nervous system malignancy: a pilot study. Neurosurgery 60(4):601–611

Nylander A, Hafler DA (2012) Multiple. Scler J Clin Invest 122(4):1180–1188

Otsuka H, Nagasaki Y, Kataoka K (2003) PEGylated nanoparticles for biological and pharmaceutical application. Adv Drug Deliv Rev 55(3):403–419

Oude Engberink RD, Blezer EL, Hoff EI et al (2008) MRI of monocyte infiltration in an animal model of neuroinflammation using SPIO-labeled monocytes of free USPIO. J Cereb Blood Flow Metab 28(4): 841–851

Papa S, Rossi F, Ferrari R et al (2013) Selective nanovector mediated treatment of activated proinflammatory microglia/macrophages in spinal cord injury. ACS Nano 7(11):9881–9895

Papa S, Ferrari R, De Paola M et al (2014) Polymeric nanoparticle system to target activated microglia/macrophages in spinal cord injury. J Control Rel 174:15–26

Park JY, Baek MJ, Choi ES et al (2009) Paramagnetic ultrasmall gadolinium oxide nanoparticles as advanced T1 MRI contrast agent: account for large longitudinal relaxivity, optimal particle diameter, and in vivo T1 MR images. ACS Nano 3(11):3663–3669

Perea G, Mriganka S, Araque A (2014) Neuron-glia networks: integral gear of brain function. Front Cell Neurosci 8, article 378

Prineas JW, Parratt JD (2012) Oligodendrocytes and the early multiple sclerosis lesion. Ann Neurol 72(1):18–31

Ransohoff RM, Cadorna AE (2010) The myeloid cells of the central nervous system parenchyma. Nature 468(7321):253–262

Reboldi A, Coisne C, Baumjohann D et al (2009) C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. Nat Immunol 10(5): 514–523

Robinson SP, Howe FA, Griffiths JR et al (2007) Susceptibility contrast magnetic resonance imaging determination of fractional tumor blood volume: a noninvasive imaging biomarker of response to the vascular disrupting agent ZD6126. Int J Radiat Oncol Biol Phys 69(3):872–879

Ruiz-Cabello J, Walczak P, Kedziorek DA et al (2008) In vivo hot spot MR imaging of neural stem cells using fluorinated nanoparticles. Magn Reson Med 60(6):1506–1511

Saleh A, Wiedermann D, Schroeter M et al (2004) Central nervous system inflammatory response after cerebral infarction as detected by magnetic resonance imaging. NMR Biomed 17(4):163–169

Schefer PW, Barak ER, Kamalian S et al (2008) Quantitative assessment of core/penumbra mismatch in acute stroke: CT and MR perfusion imaging are strongly correlated when sufficient brain volume is imaged. Stroke 39(11):2986–2992

Schmidt J, Metselaar JM, Wauben MH et al (2003) Drug targeting by long circulating liposomal glucocorticosteroids increases therapeutic efficacy in a model of multiple sclerosis. Brain 126:1895–1904

Shah L, Yadav S, Amiji M (2013) Nanotechnology for CNS delivery of Bio-therapeutic agents. Drug Deliv Transl Res 3(4):336–351

Skaat H, Corem-Slakmon E, Grinberg I et al (2013) Antibody-conjugated, dual-modal, near-infrared fluorescent iron oxide nanoparticles for antimyloidgenic activity and specific detection of amyloid-β fibrils. Int J Nanomedicine 8:4063–4076

Smirnov P (2009) Cellular magnetic resonance imaging using superparamagnetic anionic iron oxide nanoparticles: applications to in vivo trafficking of lymphocytes and cell-based anticancer therapy. Methods Mol Biol 512:333–353

Soon D, Tozer D, Altmann D et al (2007) Quantification of subtle blood-barrier disruption in non-enhancing lesions in multiple sclerosis: a study of disease and lesion subtypes. Mult Scler 13(7):884–894

Sriram S, Steiner I (2005) Experimental allergic encephalomyelitis: a misleading model of multiple sclerosis. Ann Neurol 58(6):939–945

Steinmann L, Zamvil SS (2006) How to successfully apply studies in experimental allergic encephalomyelitis to research on multiple sclerosis. Ann Neurol 60(1):12–21

Strejan GH, St Louis J (1990) Suppression of experimental allergic encephalomyelitis by MBP-coupled lymphoid cells and by MBP-liposomes: a comparison. Cell Immunol 127:284–298

Sykova E, Jendelova P (2007) In vivo tracking of stem cells in brain and spinal cord injury. Progression Brain Res 161:367–383

t Hart BA, Masacesi L (2009) Clinical, pathological and immunologic aspects of the multiple sclerosis model in common marmoset (Callithrix jacchus). J Neuropath Exp Neurol 2009 68(4):341–355

t Hart BA, van Meurs M, Brok HP et al (2000) A new primate model for multiple sclerosis in the common marmoset. Immunol Today 21(6): 290–297

Tang T, Howarth SP, Miller SR et al (2006) Assessment of inflammatory burden contralateral to the symptomatic carotid stenosis using high-resolution ultrasmall, superparamagnetic iron oxide-enhanced MRI. Stroke 37(9):2266–2270

Tofts PS, Brix G, Buckley GL et al (1999) Estimating kinetic parameters from dynamic contrast enhanced T(1)-weighted MRI of a diffusible tracer: standardized quntities and symbols. J Magn Reson Imaging 10(3):223–232

Varallyay CG, Muldoon LL, Gahramanov S et al (2009) Dynamic MRI using iron oxide nanoparticles to assess early vascular effects of antiangiogenic versus corticosteroid treatment in a glioma model. J Cereb Blood Flow Metab 29(4):853–860

Vellinga MM, Oude Engberink RD, Seewann A et al (2008) Pluriformity of inflammation in multiple sclerosis shown by ultra-small iron oxide particle enhancement. Brain 131:800–807

Weistein JS, Varallyay CG, Dosa E et al (2010) Superparamagnetic iron oxide nanoparticles: diagnostic magnetic resonance imaging and potential therapeutic applications in neurooncology and central nervous system inflammatory patholog ies, a review. J Cereb Blood Flow Metab 30(1):15–35

Wong HL, Wu XY, Bendayan R (2012) Nanotechnological advances for the delivery of CNS therapeutics. Adv Drug Deliv Rev 64:686–700

Wraith DC, Nicholson LB (2012) The adaptive immune system in diseases of the central nervous system. J Clin Invest 122(4):1172–1179

Wu X, Hu J, Zhou L et al (2008) In vivo tracking of superparamagnetic iron oxide nanoparticle-labeled mesenchymal stem cell tropism to malignant gliomas using magnetic resonance imaging. Laboratory investigation. J Neurosurg 108(2):320–329

Yednock TA, Cannon C, Fritz LC et al (1992) Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. Nature 356(6364):63–66

Yeste A, Nadeau M, Burns EJ et al (2012) Nanoparticle-mediated codelivery of myelin antigen and tolerogenic small molecules suppresses experimental autoimmune encephalomyelitis. PNAS 109(28):11270–11275

Yuan B, Zhao L, Fu F et al (2014) A novel nanoparticle containing MOG peptide with BTLA induces T cell tolerance and prevents multiple sclerosis. Mol Immunol 57:93–99

Zhang Q, Raoof M, Chen Y et al (2010) Circulating mithocondrial DAMPs cause inflammatory responses to injury. Nature 464(7285):104–107