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Nanoparticles Targeting Monocytes and Macrophages as Diagnostic and Therapeutic Tools for Autoimmune Diseases

Karen Álvarez[1] Mauricio Rojas[1,2]

[1] Grupo de Inmunología Celular e Inmunogenética, Sede de Investigación Universitaria (SIU), Universidad de Antioquia (UDEA).

[2] Unidad de Citometría de Flujo, Sede de Investigación Universitaria (SIU), Universidad de Antioquia (UDEA).

Abstract

The diagnosis and treatment of autoimmune diseases are major challenges. Their signs, symptoms, and their clinical severity change throughout the course of the disease. The available treatments for these conditions do not result in complete remission, and their long-term use generates several side effects. The main challenges in this field are the development of more sensitive diagnostic tools, and effective treatments. Studies in patients and animal models reported alterations in monocyte phenotype, activation, and function. These cells play an essential role in the pathogenesis of chronic inflammatory states and have become a target for extracorporeal monitoring and specific intervention.

Nanoparticles (NPs) are ultrafine particles that can be used as contrast agents in diagnostic imaging techniques to detect specific cells in inflammatory infiltrates in tissues that are not easily accessible by biopsy. In addition, NPs can be designed to deliver drugs to a cell population or tissue. This review describes several experimental pieces of evidence demonstrating that monocytes and macrophages can specifically uptake various types of NPs, thus becoming detectable *in vivo* by magnetic resonance imaging and susceptible to be modulated by therapeutic agents. Hence, nanoparticles targeting mononuclear phagocytes represent promising tools for diagnosing and treatment.

Key words: Autoimmune diseases, monocytes, macrophages, nanoparticles.

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1. Introduction

Autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), and multiple sclerosis (MS), are chronic conditions that result from an inadequate immune response to self-antigens (1). Diagnosis of these illnesses is challenging, as their signs and symptoms can vary widely, and their severity can change over time. It often requires a combination of several diagnostic approaches and, in some cases, very invasive procedures, such as biopsying and coronary angiography (2, 3). Regarding the treatment of autoimmune diseases, it is important to mention the current availability of various immunosuppressive therapies that help improve patients' quality of life. However, the long-term use of these therapeutic agents may cause side effects leading to progressive health deterioration. For example, systemic glucocorticoids and antimetabolites may cause renal, neurological, hematological, and immunological toxicity (4-6). In many cases, reduction of appropriate drug levels or combined therapies is required, even if the desired effects are not achieved. Therefore, the main challenges in this field are developing more sensitive diagnostic tools to facilitate early diagnosis of autoimmune diseases and more selective, timely, and effective treatments for the affected patients.

Nanoparticles (NPs) have become a promising tool for improving diagnostic and therapeutic approaches for autoimmune conditions. Nanoparticles are ultrafine particles whose three dimensions are in the nanoscale regime (generally between 1-100 nm) and have a wide variety of physicochemical characteristics in terms of size, shape, surface charge, hydrophobicity, and chemical composition(7). Nanoparticles have been widely used in biomedicine as contrast agents for diagnostic imaging methods, as platforms for drug loading and delivery, and as tools for diagnosing different conditions. They also make it possible to monitor molecular and cellular changes associated with a disease state (8, 9).

Among the different groups of NPs, the most widely used in biomedicine comprise the following (Figure 1a): (i) Lipid-based nanoparticles (liposomes, micelles, wrapsomes), which are spherical vesicles with one or more phospholipid bilayers that self-assemble in aqueous systems; they have several advantages, such as biodegradability and biocompatibility. Additionally, their amphipathic nature allows them to interact with and to transport hydrophilic compounds within the nanoparticle and carry hydrophobic compounds embedded in the lipid bilayer (6, 7); (ii) nanoparticles made from synthetic polymers such as poly(D-1-lactic acid) (PLA) and poly(D-L-lactic co-glycolic acid) (PLGA) or natural polymers such as chitosan and collagen. Given the wide diversity of polymer combinations, the variations in the composition and properties of polymeric nanoparticles are unlimited; and (iii) iron

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oxide NPs with an iron core in different oxidation states such as magnetite or maghemite; they also have a hydrophilic layer of dextran or other biocompatible components that increase their stability and functionalization and reduce their cytotoxicity. Their low toxicity, biodegradability, and ease of manufacture make them an excellent choice for drug delivery and as contrast agents for MRI. These nanoparticles are classified into different categories according to their hydrodynamic diameter, as follows: Standard (SPION; 50-180nm), ultrasmall (USPIO; 10-50nm), and very small (VSPIO: < 10nm) superparamagnetic iron oxide NPs (7) (10).

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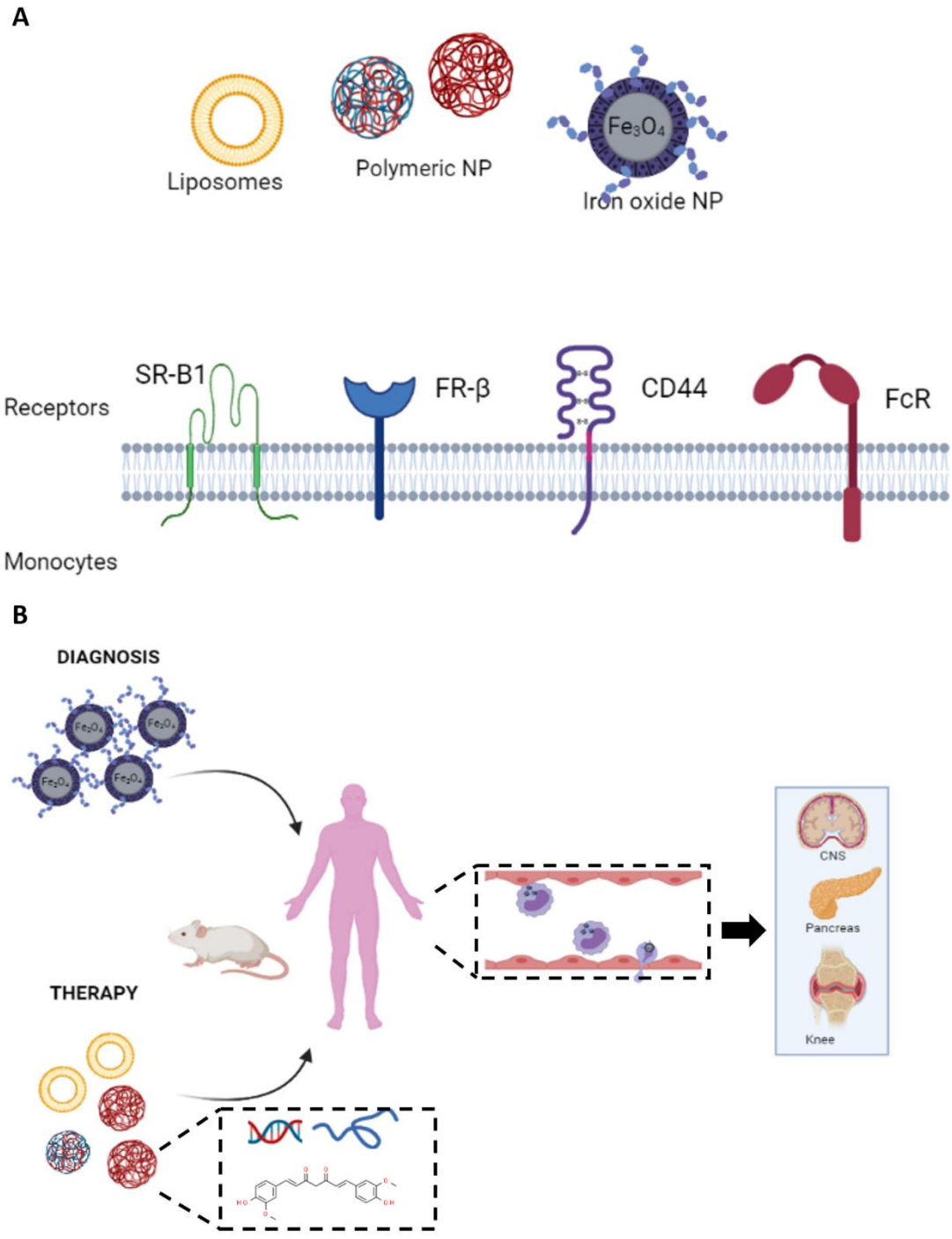

Figure 1: Monocytes and macrophages are potential targets for nanoparticles for the diagnosis and treatment of autoimmune diseases. A) According to their chemical properties, nanoparticles can be classified into different categories; liposomes, polymeric nanoparticles, and iron oxide nanoparticles are the most widely used in the diagnosis and treatment of autoimmune diseases. Nanoparticles can be

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functionalized by coating with different ligands that mediate their recognition and internalization by monocytes and macrophages through different surface receptors, including scavenger (*e.g*., SR-B1), Fc, and folate receptors. **B)** Different types of nanoparticles with superparamagnetic properties can be used as contrast agents for early diagnostic imaging of different diseases. For this purpose, nanoparticles are administered intravenously in different animal models or even in humans with autoimmune diseases to be taken up by monocytes that subsequently migrate to sites of inflammation in response to chemotactic signals. Thus, monocytes that have captured nanoparticles with superparamagnetic properties can be tracked *in vivo* by MRI to visualize their early accumulation at sites of inflammation such as the CNS (multiple sclerosis), pancreas (diabetes mellitus) or inflamed synovial tissue (rheumatoid arthritis). Furthermore, as nanoparticles can be loaded with drugs, peptides, and oligonucleotides, they can be used to specifically modulate the inflammatory function of monocytes and macrophages without compromising the function of other cells. Nanoparticles loaded with therapeutic agents increase the local concentration of the drug and reduce its side effects by lowering its systemic level.

The physicochemical characteristics of NPs are determinants of their function and interaction with different cell types. For example, the size of NPs determines their biodistribution to different tissues. NPs smaller than 20 nm in size can pass through the walls of blood vessels, the blood-brain barrier, and the epithelium of the stomach and can therefore be used as contrast agents to examine different organs by imaging. In contrast, larger NPs can be efficiently taken up by phagocytic cells. Beduneau *et al*. reported that human monocytes and monocyte-derived macrophages captured 394-nm-diameter SPIONs more efficiently than smaller 62-nm-diameter NPs (11). In addition, charge also plays an essential role in NP uptake. Juliano *et al*. demonstrated that unilamellar liposomes were cleared from mouse plasma faster than multilamellar ones. They also found that positively charged unilamellar liposomes remained in circulation longer than negatively charged ones (12). Similarly, rabbit monocytes (13) and mononuclear cells of the mouse phagocytic system (14) cleared negatively charged liposomes more efficiently than neutral ones. These results suggest that NPs with a negatively charged surface are taken up more efficiently than neutral NPs of the same size.

Encapsulation of therapeutic agents into NPs prevents them from interacting with plasma proteins, such as complement factors and immunoglobulins, and protects them against enzymatic degradation. In addition, it allows the therapeutic agent to be targeted to a specific site, thus increasing its local concentration, and reducing the side effects caused by a high circulating level or interactions with cells or tissues not involved in the event (6). The chemical nature of the surface of NPs determines

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their compatibility with immune system cells; however, they can be coated with different types of molecules to target a specific organ or cell population. In addition, NPs are a valuable tool for the intravenous administration of poorly soluble drugs that pose a significant challenge to the pharmaceutical industry as their poor aqueous solubility leads to low bioavailability and absorption. Water-insoluble drugs can be dispersed in aqueous solutions by encapsulation in NPs composed of long alternating sequences of two or more amphipathic monomers. These monomers assemble in water to form a structure with a hydrophilic corona, which can come into contact with aqueous environments, and a hydrophobic core in which water-insoluble drugs can be encapsulated.

Circulating monocytes are a subpopulation of bone marrow-derived leukocytes capable of differentiating into macrophages and dendritic cells. They constitute an essential link between innate and adaptive immunity and accomplish important immunological functions such as phagocytosis, antigenic presentation, production of soluble mediators, initiation and resolution of inflammation, and recruitment of other immune system cells. Several studies of autoimmunity in animal models and patients with autoimmune diseases have described alterations in the phenotype, function, and activation of monocytes and macrophages (15, 16). These findings have highlighted the importance of monocytes/macrophages in the immunopathogenesis of autoimmune diseases and postulated them as potential targets for improving the diagnosis of autoimmune disorders and the treatment of the affected patients. As monocytes/macrophages have endocytic and phagocytic properties, they can be targeted by ingested NPs. However, since they express a wide repertoire of receptors (scavenger receptors, Fc receptors, lectin-like receptors, integrins) and a variety of lipids and proteins, NPs could be coated with specific antibodies or other ligands to facilitate their interaction with monocytes (17).

This review describes experimental evidence on the specific uptake of NPs by monocytes/macrophages, the potential utility of NPs for *in vivo* monitoring of monocytes/ macrophages by magnetic resonance imaging (MRI), and the *in vitro* and *in vivo* modulation of the inflammatory function of monocytes/macrophages by NPs carrying different drugs, peptides, and oligonucleotides.

2. Use of nanoparticles in the diagnosis of autoimmune diseases

Early and accurate diagnosis of autoimmune diseases is necessary to treat patients properly. Nanoparticles have unique optical and physicochemical properties that allow them to be used in diagnostic procedures. For example, NPs can be utilized in molecular diagnostics to detect specific biomarkers of autoimmune diseases, such as specific DNA/RNA sequences and single nucleotide polymorphisms (SNPs) (18). Additionally, NPs can act as contrast media in MRI.

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2.1 *In vivo* imaging - animal models

In vivo imaging has become an effective approach for early diagnosis, severity assessment, and therapeutic efficacy monitoring in various chronic diseases, including autoimmune disorders. *In vivo* imaging makes it possible to localize and measure specific molecular targets and assess the physiology of a particular tissue or organ (19). In many autoimmune disorders, early inflammation is usually asymptomatic; however, it involves activation of the endothelium that, in turn, increases vascular permeability, the expression of adhesion molecules, and the tissular infiltration by different cells of the immune system (20).

Magnetic resonance imaging is a valuable method for diagnosing autoimmune diseases because it detects inflammation-associated changes such as increased vascular permeability and cellular infiltration. This procedure frequently employs different contrast media that are administered intravenously and accumulate in inflamed organs. The contrast media primarily used in daily clinical practice are based on gadolinium (Gd) and a chelating agent. Gadolinium is a heavy metal belonging to the lanthanide or rare earth family, and it is one of the most paramagnetic elements because it has many unpaired electrons. The chelating agent confers to the contrast media pharmacokinetic properties that facilitate their administration, metabolism, and elimination; and significantly decrease their toxicity, biological interactions, and deposition in tissues. Gadolinium-based contrast media transiently accumulate in inflamed tissues and enhance magnetic resonance imaging due to their ability to shorten the T1 relaxation time (21). However, they have some limitations, such as low specificity, short half-life in circulation, and association with adverse effects such as nephrogenic systemic fibrosis (19). Consequently, the development of new NP-based contrast media has been encouraged. Many studies have evaluated iron oxide NPs due to their high biocompatibility and superparamagnetic properties (22). Superparamagnetic iron oxide NPs are widely used as contrast media for MRI and are referred to as T2 or negative contrast agents. These NPs cause a magnetic field gradient that affects the surrounding protons of water molecules, thus altering the homogeneity of the magnetic field, which can be measured and observed by MRI. The Food and Drug Administration (FDA) approved the clinical use of several SPION preparations, including Ferumoxtran (dextran-SPION, 120-180 nm diameter), Ferucarbotran (carboxydextran-SPION, 45-60 nm diameter), and Ferumoxtran-10 (dextran-SPION, 20-40 nm diameter), due to their low toxicity and biodegradability (23).

Type 1 diabetes (T1D) is an autoimmune disease that results from the destruction of insulin-secreting β-cells in the pancreatic islets of Langerhans by autoreactive $CD4^+$ T cells upon recognition of one or more β-cell peptides. Throughout the onset and progression of T1D, the pancreatic microvasculature undergoes changes, such as modification of the endothelium, transient vasoconstriction, vasodilatation, and

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increased blood flow and vascular permeability. These alterations favor the infiltration of the islets of Langerhans by lymphocytes, monocytes, and macrophages. The resulting insulitis promotes β-cell death and reduces insulin production (24). An obstacle that hinders the diagnosis of T1D is the appearance of clinical manifestations when the vast majority of β cells have been destroyed, and the pancreas no longer produces enough insulin to control blood glucose levels. For this reason, efforts have been made to develop tools that make early diagnosis possible.

One of the most studied animal models of T1D is the non-obese diabetic (NOD) mouse that expresses the BDC2.5 TCR transgene (rearranged TCR α (Vα1) and β (Vβ4) chain genes) (25). These mice have autoreactive T-cells that recognize and respond uniformly to chromogranin A (ChgA), a member of the granin family that plays an essential role in hormone secretion processes. Chromogranin A is involved in the generation of insulin-containing secretory granules in the β-cells of the pancreas (26). Upon cleavage, ChgA generates several proteins, including WE-14, pancreastatin, and catestatin, which regulate carbohydrate metabolism (27). Chromogranin A-derived self-peptides are presented by H2-II Ag7 molecules to self-reactive T-cells that invade the pancreatic islets and cause insulitis. The onset and progression of insulitis are more synchronous in these TCR BDC2.5 transgenic (tg) NOD mice than in NOD mice, making it easier to follow the different stages of the disease. During the first two weeks of life, the autoimmune process is absent. However, shortly thereafter, BDC2.5^{+} T-cells abruptly invade the pancreatic islets and produce a rapidly progressive insulitis between the second and third weeks of life (24).

Magnetic resonance imaging can detect changes in the pancreatic microvasculature noninvasively because the increased vascular permeability favors the accumulation of NPs that modify the tissue contrast. Denis *et al*. used dextran-coated magnetofluorescent iron oxide nanoparticles with a long circulation time (> 10 h) to detect early changes in the pancreatic microvasculature of 4-week-old NOD mice. Following intravenous administration, NPs accumulated in the pancreas after being captured by macrophages in the tissue. The accumulation of NPs was more significant in the pancreas of NOD BDC2.5 mice than in that of Eα16/NOD (MHC-II Eα: I-Eβ) mice without insulitis. A positive correlation was found between NP accumulation and tissue injury caused by the inflammatory process (28). In 2005, a similar study reported that intravenous administration of monocrystalline superparamagnetic iron oxide NPs (MIONs; 22 nm diameter) to BDC2.5 tg NOD mice made it possible to detect inflammatory lesions *in vivo* by MRI. In addition, the study made a real-time assessment of the response of mice to treatment with a monoclonal antibody (mAb) against the CD3ε subunit of the TCR was evaluated in real time. For this purpose, 4, 8, and 18 days after initiation of immunotherapy, mice were injected with NPs, and 24 hours later, MRI was done. Monitoring of changes in

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pancreatic inflammation by MION-MRI detected responses as early as day 8 of therapy; for example, the decrease in vascular permeability was more significant in mice that had received anti-CD3 mAb than in mice treated with a control mAb. Moreover, mice treated with anti-CD3 mAb responded favorably, as evidenced by normalization of blood glucose levels within 2 to 4 weeks following treatment (29). These studies demonstrate the utility of superparamagnetic iron oxide NPs to detect early changes in the permeability of the pancreatic microvasculature associated with the onset of T1D and to monitor treatment efficacy.

Multiple sclerosis (MS) is a chronic autoimmune inflammatory disease characterized by a loss of the blood-brain barrier integrity and the development of demyelinating lesions in the brain and spinal cord that are associated with neurological axonal injury. Patients with MS present with central nervous system (CNS) inflammation characterized by infiltration of peripheral immune cells, including monocytes, neutrophils, NK-, B-, and T-cells (30). In experimental autoimmune encephalomyelitis (EAE), an animal model of MS, several phases of tissue inflammation are induced, followed by persistent myelin sheath damage in the CNS (31). This animal model can be used to design or test new therapeutic strategies for MS. The magnetic resonance imaging with Gd chelates as contrast agents has been widely used as a diagnostic tool for MS. However, Gd-MRI is limited to visualization of the secondary effects of inflammation, *e.g.*, gliosis and loss of blood-brain barrier integrity, as evidenced by diffusion of the Gd to the intercellular space.

Recently, magnetic NPs have been used as contrast agents to monitor *in vivo* the tissue distribution of phagocytes and detect for example their infiltration in the CNS. For this purpose, NPs are functionalized by conjugation with molecules that specifically bind to phagocyte membrane receptors. The NPs internalized confer sufficient magnetization to the cells to be detected by MRI or manipulated by an external magnetic field. Phagocytes internalize NPs by endocytosis or phagocytosis. However, uptake depends on the size, charge, and, mainly, surface functionalization of the NPs (32).

Inflammation in the CNS is characterized by widespread activation of mononuclear phagocytes (MPs), including both monocyte-derived macrophages and resident microglia cells. During the course of EAE, the CNS is extensively infiltrated by inflammatory monocytes and macrophages. These cells produce cytokines such as IL-1β, lL-6, and IL-23, which promote the generation and maintenance of Th17 cells. Th17 cells are crucial in the development of the CNS autoimmunity in the EAE model. Therefore, visualization of accumulated monocytes and macrophages in brain tissue is relevant. Several studies have shown that after ingesting dextran-coated USPIOs, monocytes/macrophages can be visualized by MRI in the brains of rats and mice with EAE (31, 33, 34). A subsequent study in this animal model, compared results of brain MRI scans done with different contrast media, Gd (T1-

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weighted), conventional (T2-weighted), and USPIO nanoparticles (T2-weighted). USPIO-MRI showed a higher sensitivity to detect brain lesions. It revealed the presence of tissue lesions characterized by a perivascular cellular infiltrate at sites where conventional or Gd MRI were negative. Moreover, histological analyses confirmed the presence of macrophages at sites of tissue injury detected by MRI (33). Rausch *et al.* obtained similar results in a study with the EAE model in Lewis rats immunized with guinea pig myelin. The authors demonstrated that systemically administered USPIO nanoparticles were internalized by monocytes that located in the CNS (31).

Rheumatoid arthritis (RA)is a chronic inflammatory systemic disease characterized by massive destruction of bone and cartilage and inflammation of joint synovial tissue (35). Macrophage infiltration of the synovium is one of the most important features of RA. Numerous investigations have shown that the frequency and the absolute number of macrophages are significantly increased in the affected synovial tissues of patients with RA (36). The infiltrating macrophages secrete high levels of pro-inflammatory cytokines such as TNF-α and IL-1β that participate in joint destruction by promoting the activation of synovial fibroblasts and the production of matrix metalloproteinases (37). The destructive nature of the disease, as evidenced by irreversible cartilage and bone damage, highlights the need for an early diagnosis. X-ray imaging is the primary tool for diagnosing RA and monitoring the progression of joint destruction. However, it can only detect late signs of the disease, making it difficult to prevent the irreversible bone damage in a high proportion of patients. On the other hand, MRI makes it possible to detect the early stages of RA. In this case, NPs can passively accumulate in the chronically inflamed tissue due to the enhanced permeability and retention effect. Furthermore, the diagnostic efficacy of MRI for RA can be improved by functionalizing NPs with ligands of receptors overexpressed on the membrane of the inflammatory macrophages that infiltrate the synovial tissue. In addition, *in vivo* detection of active macrophages in the affected joints by MRI is a valuable tool for monitoring the response to therapeutic agents.

Nanoparticles can be functionalized with folic acid to bind to specific cell receptors. Folate receptors (FRs) comprise a family of cell surface-anchored molecules with a high affinity for folic acid. There are three FR isoforms in humans, FR-α, FR-β and FR-γ. The FR-β receptor is overexpressed on activated macrophages that are involved in RA, Crohn's disease, SLE, and psoriasis. Dai *et al*. used the adjuvant-induced arthritis (AIA) model in Lewis rats (subcutaneous paw injection of 0.1 mL of complete Freund's adjuvant) to evaluate the detection of inflamed tissues by NP-MRI. For this purpose, they designed glucose-containing SPIONs coated with folic acid-conjugated dextran (FA glu-dex-SPIONs). These NPs detected joint inflammatory foci more effectively by targeting monocytes/macrophages that overexpressed the FR-β receptor. Moreover, *in vitro* assays with murine RAW 264.7 macrophages, previously activated with TNF-α and IFN-γ, revealed that FA glu-dex

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SPIONs were more efficiently ingested than non-conjugated NPs (38). Lutz *et al*. also demonstrated the efficacy of USPIO-MRI to detect activated macrophages in a rabbit model of RA induced by intraarticular (knee) injection of methylated bovine serum albumin. Rabbits received an intravenous injection of USPIO nanoparticles, and 24 hours later, knee images were obtained by MRI. They evidenced the uptake of the NPs by activated macrophages present in the swollen joints (37).

The findings in the animal models described above have opened the possibility of using NPs as tools to diagnose and assess the severity of inflammatory lesions in patients with autoimmune diseases.

2.2 In vivo imaging: patients with autoimmune diseases

Several studies have demonstrated the use of NPs as contrast agents for diagnosing and MRI monitoring patients with autoimmune diseases. For example, Gaglia *et al*. detected pancreatic islet inflammation by NP-MRI in patients with T1D. In this study, a cohort of 10 patients with recently diagnosed T1D (no more than six months) and 12 controls received an injection of Ferumoxtran-10 (dextran-SPION, 20-40 nm in diameter) prior to the analysis of the pancreas by MRI. The images of patients and controls were significantly different; changes in the microvasculature and infiltration of the tissue by monocytes/macrophages that had taken up the NPs evidenced the presence of inflammatory lesions in the pancreas of patients with T1D (39).

As previously described, the USPIO-MRI made it possible to visualize the cellular infiltration of the CNS of animals with EAE (31, 33, 34). For this reason, characterization of tissue lesions with NP-based contrast media has recently been implemented in patients with multiple sclerosis. The USPIO nanoparticles accumulation in phagocytic cells can reveal *in vivo* the presence of infiltrating macrophages in the brain parenchyma. Dousset *et al*. evaluated the brain lesions in 10 patients with relapsing-remitting MS by MRI. Patients received intravenous injections of USPIO nanoparticles and Gd to detect different aspects of the CNS inflammatory process. The uptake of the nanoparticles by circulating monocytes made it possible to visualize their activity *in vivo* and their presence in the CNS cell infiltrate. On the other hand, the Gd revealed the increased permeability of the blood-brain barrier (40). Furthermore, some experimental evidence suggests that, compared to the Gd, the USPIO nanoparticles confer greater sensitivity to MRI in detecting the number of CNS lesions (41, 42). In 2008 Vellinga *et al*. studied 19 patients with relapsing-remitting MS and observed that in 14 individuals with active disease, MRI showed 188 USPIO-positive lesions, of which 144 were Gd negative (41). Additionally, Tourdias *et al*. demonstrated that the combination of both contrast agents (USPIO nanoparticles and Gd) helped to detect lesions that were missed when Gd was used alone (increased detection rate of 51%) (42). The additional information provided by NPs to Gd-enhanced MRI may be related to monocyte

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infiltration of the CNS. Therefore, USPIO-enhanced MRI could provide a better understanding of the complexity of the inflammatory process in multiple sclerosis.

2. Use of nanoparticles in the treatment of autoimmune diseases

The treatment of autoimmune diseases is focused on controlling the immune response, either with immunosuppressive drugs that induce antigen tolerance, or by enhancing the activation of the immune response. The potential of NPs has been evaluated in both scenarios.

3.1 Nanoparticles as drug carriers-Animal models

The use of biodegradable nanoparticles to transport different drugs, peptides, and oligonucleotides has been extensively evaluated *in vitro* and *in vivo.* These particles have several advantages, such as biocompatibility, increased cargo uptake rate, and sustained cargo release. They also make it possible to overcome biological barriers, thus favoring the delivery of the cargo to the target cells, tissues, or organs at the appropriate concentrations.

The FDA has approved several drugs for treating patients with MS, such as glatiramer acetate, dimethyl fumarate, natalizumab (anti-VLA-4), and alemtuzumab (anti-CD52 antibody). However, all of them are associated with different side effects. One of the main obstacles to treating patients with diseases of the CNS is the inability of most therapeutic agents to cross the blood-brain barrier. Therefore, nanoparticle-based strategies are under development to facilitate the passage of molecules across it (43, 44).

As described above, monocytes play an essential role in the onset and evolution of MS because they cross the blood-brain barrier, promote neuronal damage, and recruit other immune system cells into the CNS. Lu *et al*. injected EAE mice with synthetic high-density lipoprotein-mimicking peptide-phospholipid scaffold nanoparticles (HPPS) loaded with curcumin (cur) to evaluate their immunomodulatory activity on inflammatory monocytes. They found that monocytes ingested cur-HPPSs through the scavenger SR-1 receptor in both *in vivo* and *in vitro* experiments. Mice treated with cur-HPPSs showed lower morbidity and mortality than those that received free curcumin. These results were attributed to the ability of cur-HPPS to modulate inflammatory monocytes by inhibiting the NF-κB pathway and decreasing the expression of adhesion and migration-related molecules, such as ICAM-1 and Mac-1 (43). Also, in the EAE mouse model, Saito *et al*. evaluated drug-free nanoparticles synthesized from 50:50PLG (poly DL-lactide-co-glycolide) with low or high molecular weight or poly (D-L-lactide) (PLA) with low molecular weight (PDLA). Specifically, they assessed the ability of these NPs to associate with circulating monocytes and neutrophils to prevent them from migrating to the CNS and their impact on the course of the disease. *In vitro* and *in vivo* experiments

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showed that neutrophils and monocytes effectively took up the NPs. Furthermore, intravenous treatment with PLG-H nanoparticles induced a significant reduction in the disease score and the number of myeloid cells and $CD4^+$ T-cells in the CNS (45). These findings demonstrated that NPs are a promising tool to target cells of the innate immune system, such as inflammatory monocytes, and reduce their traffic to tissues injured or inflamed.

Regarding RA, therapeutic agents are intended to reduce joint inflammation and damage. However, not all patients respond effectively to these drugs; some have adverse side effects, such as hair loss, headache, and lung, liver, and kidney toxicity. Therefore, several studies have evaluated the therapeutic potential of NPs to target the drug specifically to the cells involved in the inflammatory process (46, 47). Since macrophages infiltrate the inflamed synovial membrane and cartilage and play a critical role in RA pathogenesis, the design of NPs that specifically target these cells has been widely considered. Thomas *et al*. demonstrated *in vitro* that RAW 264.7 macrophages and macrophages isolated from the peritoneal cavity of mice efficiently uptake methotrexate encapsulated in polyamidoamine dendrimers (PAMAM) covalently conjugated with folic acid. Methotrexate loaded NPs were recognized by FR-β receptors, whose expression is selectively elevated in synovial macrophages of patients with RA. They also demonstrated the therapeutic potential of MTX-NPs when administered intravenously to rats with collagen-induced arthritis (CIA). A favorable response was evidenced by the significant reduction in ankle diameter, paw weight, and total body weight in rats with CIA compared to animals given free methotrexate (46). In 2020, another study evaluated the characteristics and therapeutic potential of folate-conjugated chitosan-glycol nanoparticles (FGCNs) loaded with MTX (MFGCNs) in the Wistar rat model with AIA. Such NPs were stable in serum and did not induce hemolysis of rat red blood cells. Lipopolysaccharide (LPS)-activated murine RAW 264 macrophages took up MFGCNs via FR-β to a greater extent than non-activated macrophages; human embryonic kidney (HEK) cells and mouse embryonic fibroblasts (NIH-3T3) did not take up the NPs. Interestingly MFGCNs ingested by macrophages induced reactive oxygen species (ROS) production and reduction of antioxidant enzymes that led to cell apoptosis (47). Additional *in vivo* experiments showed that, after intravenous administration of ^{99m}Tc radiolabeled MFGCNs to AIA rats, the NPs accumulated in high concentrations in arthritic joints. Furthermore, NPs induced a reduction in ankle diameter, paw thickness, and arthritis score that were superior to those observed in rats injected with free MTX or phosphate buffered saline (PBS) (47). These results suggest that specific uptake of MTX-loaded NPs by inflammatory macrophages through FR-β receptors can suppress the inflammatory changes associated with arthritis.

Another study described the therapeutic properties of mineralized NPs (MN-HANPs) composed of PEGylated hyaluronic acid (P-HA) as a hydrophilic layer, 5β-cholanic acid as a hydrophobic core, and calcium phosphate (CaP) as a pH-sensitive mineral.

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These particles can release a cargo across neutral to acidic conditions as those of inflamed joints. *In vitro* assays showed that macrophages internalized these particles through endocytosis mediated by different molecules, mainly CD44, stabilin-2, and the receptor for hyaluronan-mediated motility (RHAMM). In addition, MN-HANPs were loaded with MTX to verify drug release at different pH conditions (from 7.4 to 5.0); the drug release was higher as the pH decreased. Moreover, symptom relief was observed when MN-HANPs were conjugated with MTX and injected into mice with CIA (48). Thus, transport of MTX on NP-based platforms targeting sites of inflammation could reduce drug-associated side effects.

3.2 Nanoparticles as carriers for nucleic acids

Gene silencing with siRNA has been explored as a therapeutic strategy for the treatment of different diseases (49). However, the instability of siRNA under physiological conditions limits its therapeutic use. Nanoparticles have become an alternative for docking and transporting siRNA molecules to increase their stability and prevent their degradation by circulating nucleases. Howard *et al*. reported that intraperitoneal administration of chitosan NPs loaded with siRNA targeting the gene encoding TNF-α (siRNA-TNF-α) to mice with CIA reduced TNF-α production by peritoneal macrophages and induced a reduction in local and systemic inflammation (50). Similarly, a study published in 2012 demonstrated that PLGA NPs loaded with siRNA-TNF-α inhibited TNF-α expression in RAW 264.7 macrophages in a dose-dependent manner. Furthermore, administration of these NPs to DBA/1J mice with collagen antibody-induced arthritis (CAIA) induced a reduction in paw inflammation and joint effusion (51), thus demonstrating the therapeutic potential of siRNA-loaded NPs.

Since inflammatory monocytes depend on the chemokine receptor CCR2 to migrate to foci of inflammation, Leuschner *et al.* designed 70-80 nm lipid NPs containing siRNA-CCR2 and studied their effect after intravenous administration in mice with different inflammatory conditions (atherosclerosis, myocardial infarction, pancreatic islet transplantation in diabetes, and cancer induced by EL4 lymphoma cell implantation). The CCR2 knockdown was achieved in Ly-6C^{high} monocytes isolated from the spleen and was confirmed at the mRNA and protein levels. In addition, intravenous injection of siRNA-CCR2-NPs to mice decreased monocyte migration to foci of inflammation and thus produced favorable effects in the different models studied: (i) attenuated the number of atherosclerotic plaques, thus reducing the myocardial infarct size after coronary artery occlusion; (ii) prolonged normoglycemia in diabetic mice after pancreatic islet transplantation; and (iii) reduced tumor volume and the number of tumor-associated macrophages (TAMs) in the EL4 lymphoma model; interestingly, TAMs inversely correlates with survival in patients with lymphoma (52).

3.3 Nanoparticles as drug carriers - Human monocytes

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Although the FDA has accepted the clinical use of some nanoparticles, such as SPIONs, more experimental evidence is still needed for other nanoparticles to be used as therapeutic tools to treat patients with different diseases. As the design of new nanoparticles progresses, it becomes necessary to evaluate their effect on human cells. Several studies have shown how nanoparticles interact with and affect different human cells, including some of the immune system, such as lymphocytes, monocytes, macrophages, and dendritic cells. The preponderant role of monocytes and macrophages in the onset and progression of autoimmune diseases has made them attractive targets for diagnosing and treating these conditions. On the other hand, the interaction of NPs with circulating proteins, such as opsonins and other blood components, favors their recognition and internalization by monocytes and macrophages (53). These interactions also play a fundamental role in the biodistribution of NPs since, after being ingested by monocytes that migrate to infected or inflamed tissues, the NPs can accumulate to a greater extent in these compromised areas (54).

In order to develop contrast media for selective and differential tracking of human monocytes *in vivo*, Giraldo-Villegas *et al*. evaluated the features of polyacrylate-coated iron oxide nanoparticles (PAC-IONs). They found that PAC-IONs were captured by monocytes through scavenger receptors and did not affect their viability, differentiation to macrophages, or ability to phagocytose latex spheres (55). Since monocytes efficiently capture PAC-IONs without adverse effects on their phenotype and function, they could be a valuable tool for characterizing early tissue injury and understanding their role in different immunopathologies, including autoimmune diseases.

Chitosan NPs have also been explored for targeting monocyte/macrophages. Chitosan is a natural and biocompatible polymer that has been extensively studied in various biomedical applications, such as drug delivery and tissue engineering strategies. As chitosan could interfere with the phenotype and function of some cells, it is critical to understand its effect on macrophage characteristics. In 2012 Oliveira *et al*. evaluated for 10 days the polarization of human monocyte-derived macrophages on an ultrathin chitosan film and observed a reduced expression of the surface molecules CD86 and HLA-DR, reduced production of proinflammatory cytokines such as TNF-α, and increased production of the anti-inflammatory cytokines IL-10 and TGF-β1. These results suggested that chitosan induces the polarization of macrophages towards the anti-inflammatory-M2 phenotype (56). Subsequently, in 2015, the same group designed chitosan and γ-glutamic acid (γ-PGA) NPs loaded with diclofenac (Df-NPs) and evaluated their effect on human macrophages. These NPs were efficiently taken up by macrophages and inhibited prostaglandin E2 synthesis (57), *i.e*., they showed anti-inflammatory activity.

Another study by Rafique *et al.* described the design of PEG-coated lipid NPs containing calcitriol and functionalized with anti-CD163 antibodies (PEG-LNP(Cal)-αhCD163) and evaluated their effect on human monocyte-derived macrophages. The particles were endocytosed by macrophages through the CD163 receptor and induced the reduction of NF-κB, TNF-α, MCP-1, and IL-6 mRNA levels; a lower

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secretion of TNF-α and IL-6; and the increase of IL-10 mRNA levels (58). These data demonstrated that drug-loaded NPs could contribute to modulating the inflammatory function of monocytes and thus ameliorate local inflammatory reactions typical of autoimmune diseases.

3.3.1 Nanoparticles and monocyte subpopulations

At least three monocyte populations have been described in humans based on surface expression of CD14 (LPS co-receptor along with MD-2 and TLR4, which mediates LPS signaling) and CD16 (FcγIIIa receptor) as follows: classical ($CD14^{++}/CD16^{-}$), intermediate ($CD14^{+}/CD16^{+}$) and non-classical ($CD14^{+}$, $CD16^{++}$) monocytes. Several authors have described striking alterations in the proportion and the absolute number of circulating monocyte subpopulations in a wide range of patients with different diseases, including autoimmune diseases such as SLE (59, 60) and RA (61). Therefore, several studies have sought to elucidate the ability of different monocyte subpopulations to internalize nanoparticles and their consequent effects. For instance, Settles *et al*. showed by flow cytometry that classical monocytes, isolated from human peripheral blood, took up superparamagnetic fluorescent iron oxide nanoparticles more efficiently than non-classical monocytes. Furthermore, fluorescence microscopy assays showed that the uptake of NPs was clathrin-dependent, as evidenced by the colocalization of fluorescent signals from clathrin vesicles and particles. Internalized nanoparticles altered the monocyte phenotype: classical monocytes showed increased expression of CCR2 and CD120a; non-classical monocytes exhibited increased expression of CD206 and decreased expression of the fractalkine receptor CX3CR1, and both cell subsets showed a higher expression of HLA-DR (62).

Wildgruber *et al*. compared the uptake of dextran-coated superparamagnetic iron oxide NPs coupled to macrophage colony-stimulating factor receptor (MCSFR) antibodies by classic and non-classic monocytes. They found that, although both cell subpopulations expressed similar levels of MCSFR, the uptake of NPs was better in classical monocytes and did not affect their viability (63). This differential uptake of NPs by monocyte subpopulations could be used for in vivo detection and characterization of these cell subsets in different diseases.

Conclusion

Monocytes are a population of bone marrow-derived leukocytes that in response to chemotactic signals migrate to sites of inflammation, where they can differentiate into macrophages and dendritic cells. All these cells accomplish several essential immunological functions, including phagocytosis, antigenic presentation, production of soluble mediators, initiation and resolution of inflammation, as well as recruitment of other cells of the immune system. Monocytes/macrophages play an essential role in the onset and progression of different autoimmune diseases, including SLE, RA, and MS. Moreover, these cells can efficiently internalize different types of NPs, cross

nearly impermeable biological barriers, and migrate early to foci of inflammation. Due to these characteristics, several research groups have proposed that monocytes/macrophages be used as targets of superparamagnetic nanoparticles for in vivo monitoring by MRI. Additionally, as NPs can be loaded with some drugs, peptides, and nucleic acids, they can be used not only to visualize the location of monocytes/macrophages in vivo but also to precisely modulate their inflammatory function. The experimental evidence described in this review supports the potential use of nanoparticles in the early diagnosis and effective treatment of autoimmune diseases (Figure 1).

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