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Harnessing immunomodulatory mechanisms of *Trichinella spiralis* to design novel nanomedical approaches for restoring self-tolerance in autoimmunity

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ABSTRACT

The rapid increase in the prevalence of autoimmune diseases in recent decades, especially in developed countries, coincided with improved living conditions and healthcare. Part of this increase could be ascribed to the lack of exposure to infectious agents like helminths that co-evolved with us and display potent immune regulatory actions. In this review we discussed many investigations, including our own, showing that *Trichinella spiralis* via its excretory-secretory products attenuate Th1/Th17 immunopathological response in autoimmunity and potentiate the protective Th2 and or regulatory T cell response, acting as an effective induction of tolerogenic dendritic cells (DCs), and probably mimicking the autoantigen in some diseases. A recent discovery of *T. spiralis* extracellular vesicles (TsEVs) suggested that inducing a complex regulation of the immune response requires simultaneous delivery of different signals in nano-sized packages. Indeed, different artificial nanomedical approaches discussed here suggested that co-delivery of multiple signals via nanoparticles is the most promising strategy for the treatment of autoimmune diseases. Although a long way is ahead of us before we could completely replicate natural nano-delivery systems which are both safe and potent in restoring self-tolerance, a clear path is being opened from a careful examination of parasite-host interactions.

1. Introduction

The prevalence of autoimmune diseases increased in recent decades, especially in developed countries, which represents a large health-related socio-economic and societal challenge. This rapid increase cannot be explained by genetic changes, but is, most likely, influenced by changes in the environment that could cause improper regulation of the immune system [1], which leads to adverse inflammatory responses and the loss of self-tolerance. Treatment that would restore the balance of the immune system, thereby influencing the mechanisms underlying the disease initiation and development, would be a major advance over existing disease-modifying therapies.

Given that there is no cure for autoimmune diseases and that currently available therapies cannot restore self-tolerance and provide only temporary remission, we put our efforts into designing therapeutic approaches that will result in triggering immune regulatory mechanisms, reflected in the induction of regulatory T cells (Tregs), regulatory B cells (Bregs), tolerogenic dendritic cells (tolDCs) and other myeloid regulatory cells, as well as anti-inflammatory and regulatory cytokine production, in the aim to restore tolerogenic immune response and silence already established autoimmune processes. Induction of tolerogenic functions was accomplished by *Trichinella spiralis* infection, its excretory-secretory products, or its extracellular vesicles (EVs), as well as by various nanomaterials. The combination of the most potent products from *T. spiralis* and their most efficient delivery via nanomedical approaches seems the most promising strategy for the treatment of autoimmunity diseases and hope for the suffering patients.

1.1. *Trichinella spiralis and autoimmune diseases*

Helminths coevolved with humans and are thought to be involved in the development of immune regulatory mechanism, necessary for keeping a balanced immune response [2]. Once spread all over the globe, helminths almost completely vanished from the developed countries due to improved living conditions and health care, which coincided with the increased incidence of autoimmune diseases in the

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last decades of the 20th century [3]. It is assumed that lack of exposure to infectious agents, like helminths, is one of the causes for dysregulation of the immune system and consequently increased susceptibility to immune-mediated diseases, such as multiple sclerosis (MS), diabetes mellitus type 1 (T1D), and rheumatoid arthritis (RA) [4]. A concept of helminth therapy in a variety of autoimmune disease exists for quite some time, and a large body of epidemiological, experimental, and clinical data goes in favor of the hypothesis that helminth infections influence the immune system in a way that could enable protective pathways against the autoimmune diseases [4]. Since the usage of the infection itself raises many safety and ethical issues, the potential of helminth products to ameliorate autoimmune diseases has been explored to develop a new therapeutic approach for inflammatory disorders [5-7].

Many studies are suggesting that *T. spiralis* is a good candidate for induction of immunomodulation that could result in alleviation of symptoms of different autoimmune diseases like experimental colitis as a model of inflammatory bowel disease (IBD), experimental autoimmune encephalomyelitis (EAE) as a model of multiple sclerosis (MS), experimental diabetes mellitus type 1 (T1D), and experimental rheumatoid arthritis (RA) [8]. *T. spiralis* infection exerts its long-lasting impact on the host immune system through its excretory-secretory products (ES L1) released from muscle larvae encapsulated in a transformed muscle cell, called nurse cell [9]. Besides induction of Th2 immune response which dominates over Th1 in *T. spiralis* infection, ES L1 products activate regulatory responses which suppress the host immune response against the parasite itself to survive, but also mitigates the unwanted immune responses, like those to autoantigens [8,10]. The complex interplay between the parasite and the host immune system provides an overwhelming source of potential immunomodulatory mechanisms which could be harnessed for modulating autoimmunity.

In a mouse model system of colitis, induced by trinitrobenzesulfonic acid (TNBS) and oxazolone (OXZ), *T. spiralis* muscle larvae, administrated upon chemical induction of the disease, successfully increased the survival rate of mice and lessen the gut tissue damage. Reduced granulocyte infiltration, as well as overall lower macroscopic and histological damage score resulted in the recovery of gut mucosa [11]. The authors suggested that the increase in IL-4 and IL-10 induced by *T. spiralis* infection, and consequent delay in IFN-γ and IL-2 increase, both in the target tissue and on the systemic level, are responsible for the lower tissue damage observed in *T. spiralis*-infected mice with colitis. In addition to therapeutic, other authors examined the prophylactic effect of infection on the development of colitis in an animal model. Several studies indicated that *T. spiralis* infection can mitigate the severity of the disease more profoundly if it preceded the induction of colitis, regardless of the mode of disease induction (TNBS or acetic acid). When the colitis was induced by TNBS [12], the authors showed the decrease of disease score, as well as microscopic and macroscopic colon damage, an increase of IL-4, lower IFN-γ expression, and lower expression of NF-kB in colon tissue, accompanied by increased percentage of CD4+CD25+Foxp3+ Tregs on local and systemic levels. These results implied that pre-infection with *T. spiralis* balanced the Th1 type of intestinal inflammation by the induction of Th2 and regulatory type of immune response. Another study performed by Ashour et al. [13] showed that the amelioration of acetic acid-induced colitis was related to prophylactic *T. spiralis* induced recruitment of Foxp3+ Tregs to the areas of inflammation. In contrast, the recent study of Zheng et al. [12] on experimental colitis induced by dextran sodium sulfate (DSS) showed that the lower inflammation rates, increased level of regulatory cytokines (IL-10 and TGF-β), but down-regulated Th1 and Th2 responses were responsible for the therapeutic effects of *T. spiralis* infection and that this approach has a better potential to restrain the intestinal inflammation in experimental colitis compared to the prophylactic treatment. Altogether it seems that both prophylactic and therapeutic approaches are plausible for *T. spiralis* infection, but the underlining mechanisms and the overall immunological effects could differ depending on the mode of colitis induction, the dose of infection, animal model and, depending on the genetic background, even the animal strain used in the study.

Another model of autoimmune disease that has been quite frequently used for the research of the capacity of *T. spiralis* infection to modulate the course of the disease is EAE. First results on this phenomenon have been reported by our group [13] demonstrating that the infection with *T. spiralis* ameliorates EAE in a model system of Dark Agouti (DA) rat in a dose-dependent manner. Namely, modulation of the severity of EAE by the infection with 500 muscle larvae was reflected in the lower cumulative index and maximal severity score, as well as in the reduction of illness duration and the number of mononuclear cells infiltrations in the central nervous system (CNS). *T. spiralis* infection introduced before the induction of EAE increased the levels of Th2 and regulatory cytokines (IL-4 and IL-10) and decreased Th1 and Th17 responses in draining lymph nodes [14]. Protective effects of *T. spiralis* infection in EAE were also confirmed by transferring splenic T cells from *T. spiralis* infected rats into EAE immunized rats without infections, implicating that T cells, particularly IL-10-producing CD4+CD25+Foxp3+ T regs, were predominantly responsible for the observed suppressive effects in EAE. So, prophylactic application of *T. spiralis* infection showed that this parasite can create an unfavorable environment for the development of EAE by Th2-switch and induction of regulatory immune response [13-15].

The potential of *T. spiralis* infection to attenuate the severity of autoimmune disease was also demonstrated on the model of collagen-induced arthritis (CIA) in mice. It was shown that the effect of infection, reflected in the induction of Th2 bias and activation of a regulatory T cell response, is dependent on programmed death 1 (PD-1), as an important inhibitory receptor engaged in the balance of host immune responses [16]. On the other hand, the study of Osada et al. [17] has shown that *T. spiralis* capacity to mitigate CIA and its potential therapeutic application is independent of Th2 response and that infection provides anti-arthritic effects by other mechanisms, implicating that the regulatory arm of immune response is more important in restraining CIA then Th2 response itself. Additionally, *T. spiralis* infection effect on the course of T1D in NOD mice was studied upon the infection with this helminth and the results revealed that the infection alters the severity of T1D by Th2 immune response bias which proved to protect the mice from the response leading to beta cells destruction [18].

In general, studies on the effect of *T. spiralis* infection on mitigation of different autoimmune disorders on animal model systems showed that the balanced immune response provoked by this infection i.e. Th2 slight predominance over Th1, as well as the engagement of the regulatory T cells, provided the environment which prevents autoimmunity. Although tempting, the deliberate prophylactic or therapeutic infections with *T. spiralis* in clinics are neither ethical nor medically justified due to risks of complications and potential adverse effects. Therefore, careful evaluation of the mechanisms utilized by *T. spiralis* to modulate autoimmunity opens the perspective to their safe and controlled usage in clinics.

1.2. T. spiralis effects in autoimmune diseases and its potential mechanisms

The application of total ES products (originating from muscle larvae or adult parasites), as well as individual components (in native form or recombinant), showed that they exert strong immunomodulatory effects and can act prophylactically or therapeutically on animal models of autoimmune diseases [2]. *T. spiralis* infective muscle larvae (L1) have a critical role in the invasion of the host, establishment, and maintenance of the parasitism, as well as in the induction of the host's immune response [8]. Hence, the products of chronic, muscle life-cycle stage of the parasite, ES L1, are in the focus of several studies relating to the potential of *T. spiralis* products to reproduce the effect of the infection itself in the alleviation of autoimmune diseases. ES L1 products represent a complex mixture of 43 glycoproteins, identified as 13 proteins and

their isoforms which occurred due to post-translational modifications and protein processing [19]. ES L1 contains proteinases and their inhibitors, heat shock proteins, kinases, phosphatases, glycosidases, endonucleases, DNA-binding proteins, and others [8]. Some of the molecules within ES L1 have been investigated in sense of their importance for the activation of immune response characteristics for *T. spiralis* infection. Proteins of 45, 49, and 53 kDa, that bear the immunodominant epitope, unique for the muscle larvae of the whole genus *Trichinella* are recognized in Western blot by sera obtained from different *T. spiralis*-infected species (humans, pigs, dogs, horses, and rats) [20]. Components of this glycoprotein triplet, together or individually (as 53 kDa protein), are good candidates for the creation of anti-inflammatory milieu and modulation of autoimmune diseases since it has been shown that they can largely reproduce the effect of the native ES L1 on the phenotype and function of DCs [21]. The excretory-secretory products of the adult life stage (AES) of *T. spiralis* also represent a heterogeneous group of proteins, some of which might be considered as early diagnostic markers of trichinellosis [22]. Components of AES recognized by sera of patients with trichinellosis or from animals with *Trichinella* infection may contain antigens responsible for triggering the host immune system. So far, the capacity of the native *T. spiralis* products derived from adults and muscle larvae, and some of their components to modulate the course and severity of autoimmune diseases have been explored in several model systems.

A few studies pointed out that AES exhibit a therapeutic potential to mitigate DSS-induced colitis in mice in sense of Th2/Treg cytokines increase in gut-associated lymphoid tissue (GALT), mesenteric lymph nodes, and spleen [23,24]. On the other hand, a total soluble antigen of *T. spiralis* muscle larvae had a beneficial prophylactic effect on TNBS-induced colitis if applied locally (rectal submucosal administration) i.e. myeloperoxidase activity was down-regulated, as well as IL-1β and iNOS expression, while the production of Th2 and Treg cytokines (IL-13 and TGF-β) were elevated [25]. In addition to whole *T. spiralis* products either from adults or muscle larvae, which provided evidence on the potential of a complex mixture of antigens to inhibit experimental colitis in mice, isolated components with a role in the invasion of the host immune response and parasitism establishment, particularly proteinase inhibitors present both in *T. spiralis* adults and muscle larvae antigens, were individually tested for immunomodulatory capacities. Namely, *T. spiralis* cysteine proteinase inhibitor in a form of recombinant protein (TsCystatin) showed beneficial prophylactic effects on TNBS-induced colitis by reducing microscopic and macroscopic damage in colon tissue, decreasing IFN-γ and NF-κB expression while increasing IL-4 [26]. Since the percentage of CD4+CD25+Foxp3+ T cells wasn't elevated on a systemic level, the authors concluded that TsCystatin alleviates experimental colitis through the induction of Th2 type of immune response, thereby opposing the pathogenic effects of Th1-mediated inflammation. The same impact on alleviation of experimental colitis, with the addition of significant CD4+CD25+Foxp3+ Treg population role in the phenomenon, was observed upon prophylactic treatment of mice with both Kazal-type and adult type of *T. spiralis* serin protease inhibitors (serpins), which are engaged in parasites' evasion of host's immune mechanisms [27], and the phenomenon was confirmed by using recombinant *T. spiralis* adult serin protease as well [28]. One of the potentially protective agents for IBD, experimental colitis mouse model, is 53 kDa immunodominant component of muscle larvae products - ES L1, which induce strong Th2 response and IgG1 type of antibodies. Recombinant 53 kDa protein reduced the disease activity index, microscopic and macroscopic score, decreased local and systemic levels of Th1 cytokines (IFN-γ, THF-α) while increasing the systemic level of Th2 cytokines (IL-4, IL-13) and regulatory cytokines (IL-10, TGF-β) in colon tissue [29].

Immunomodulatory properties of *T. spiralis* products were demonstrated in the EAE model system, either used alone or via treated DCs. Total soluble muscle larvae extract decreased the production of Th1 cytokines (TNF-α and IL-12) in DCs activated with type-1 stimulus, while increased the expression of OX40L, which most contributes to the development of Th2 and regulatory immune responses [30]. The observed mechanisms were probably involved in the suppression of symptoms in the mouse EAE model by prophylactic application of ES L1. Our group has shown that ES L1 induces semi-matured DCs phenotype which displays a low capacity for IL-12 production and an increased capacity to produce IL-10, all of which proved to be a successful prophylactic toll in the amelioration of EAE in the DA rat model system [31]. Rats with EAE, induced upon the application of ES L1-treated DCs, exhibited lower production of IL-17 and IFN-γ, and elevated production of IL-4, IL-10, and TGF-β both on a systemic level and in CNS as target tissue. The increased percentage of CD4+CD25+Foxp3+ Treg cells on the local and systemic level, together with elevated IL-10 and TGF-β are likely to be the key players in restraining the immune response that leads to autoimmunity. We also investigated the capacity of ES L1 itself to modulate the severity of EAE if administrated prophylactically and demonstrated that ES L1 induces the shift of immune response towards Th2 type both on the local and systemic level, as well as the activation of the regulatory arm of response, but surprisingly, the majority of Tregs did not express CD25 [32]. The important finding of that study is the potential of ES L1 *in vitro* to modulate the activity of existing autoreactive T cells, as well as to induce anti-inflammatory phenotype of MOG-pulsed DCs, implying that ES L1 can act therapeutically by modulating both innate and adaptive immune cells. In a human model of DCs, we found that ES L1 induces tolDCs by engaging TLR2, TLR4 [33] as well as DC-SIGN [34]. The effects of ES L1 on up-regulation of CD40 and CCR7 required only DC-SIGN, whereas all three receptors were involved in the induction of Th2 and Treg, suggesting that ES L1 provides unique tolerogenic signals for the host immune system via DCs (Fig. 1). Hence, *T. spiralis* ES L1 presents a potent immunomodulator of EAE course and severity, with the potential for prophylactic and therapeutic use.

1.3. Extracellular vesicles as biological nanostructures for modulation of immune response by T. spiralis

To harness the full potential of *T. spiralis* in the therapy of autoimmune diseases it is necessary to investigate all its components involved in mechanisms of its immunomodulatory effects. A recent discovery that helminths produce EVs and that EVs are part of helminths' excretory-secretory products (ES) [35] shed new light on the mechanisms by which *T. spiralis* deliver information to the host immune system. EVs are nano-sized membrane-enclosed vesicles (30-1000 nm) released from all cells to the intercellular space [36]. They can originate from the endosomal compartment of the cell, starting as 'intraluminal vesicles' within the multivesicular body (MVB) and being released as 'exosomes', upon fusion of MVB and plasma membrane. The second type of EVs are formed by budding of the plasma membrane and are designated as 'microvesicles' upon detachment. Exosomes are generally smaller than microvesicles and different populations of EVs carry different assemblies of molecules. However, since both size and composition may overlap between different types of EVs and currently available techniques do not allow their absolute separation, the International Society for Extracellular Vesicles (ISEV) recommends the use of the term EVs [37]. EVs can carry all types of biomolecules (proteins, lipids, RNA, fragments of DNA, metabolites, small molecules) which are packaged in a selective process and can be carried either as luminal cargo or can compose integral membrane constituents [36]. EVs released from one cell can interact with proximate cells or be transported by biofluids to distant cells (i.e. in different organs). Such interaction implies the transfer of bio information and causes a change in the physiology of target cells. Since all cells use this mechanism, EVs represent the third fundamental mode of intercellular communication, along with direct cellular contact and exchange of soluble molecules. In the last decade, numerous studies showed EVs' involvement in all (patho)physiological processes in all organisms [36]. Furthermore, EVs are involved in communication

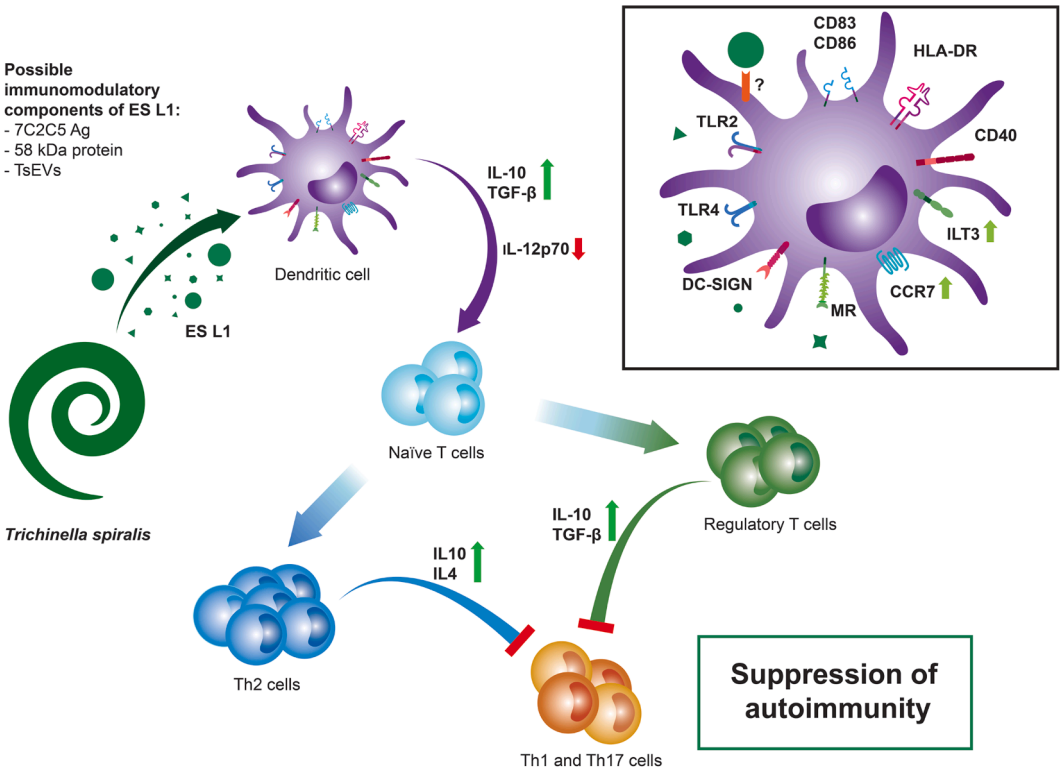

Fig. 1. Potential mechanisms of restoring self-tolerance by *T. spiralis* muscle larvae excretory-secretory (ES L1) products. ES L1 components with immunomodulatory potential induce semimatured DCs, with low capacity for IL-12 production and an increased capacity to produce IL-10. These cells are capable of naïve T cell polarization towards Th2 and regulatory type of responses. Induced Tregs and elevated IL-10, TGF-β and IL-4 release impair Th1/Th17 response development and suppress autoimmune diseases (Design AGM, Artwork MK).

between different organisms and even different species [36]. Such is the case of EVs-mediated interaction of parasites and their hosts [35].

Helminths generally produce EVs as part of their excretory/secretory products but in the case of Platyhelminthes, EVs could be also released from the tegument [35,38]. Both larval and adult stages of helminths were found to release EVs [39]. EVs were found to be produced by flukes *Fasciola hepatica* [38], *Echinostoma caproni* [40], *Schistosoma mansoni* [41], and *S. japonicum* [42], *Opisthorchis viverrini* [43], and *Dicrocoelium dendriticum* [44]. EVs are also produced by cestodes, i.e. *Echinococcus granulosus* [45]. Among nematodes, *Heligmosomoides polygyrus* [46], *Trichuris suis* [47] and *T. muris, Teladorsagia circumcincta* [48], *Haemonchus contortus* [49], *Brugia malayi* [50] and *Ascaris suum* [51] are all found to produce EVs. EVs released by parasites are involved in host-parasite communication [52,53]. It was shown that EVs of *E. caproni* and *H. polygyrus* are internalized by rat intestinal cells [38,46]; murine macrophages internalize *B. malayi* EVs [50]; murine colonic organoids can take up EVs from *T. muris* [54]; mice macrophages internalize EVs from *H. polygyrus* [55]; DCs take up *E. granulosus'* EVs [56]; EVs from *O. viverrini* are taken up by cholangiocytes [57] while EVs from *S. mansoni* are taken up by both DCs [58] and endothelial and monocytic cell lines [39]. Interaction of helminths' EVs with host cells influences their physiology and may cause immunomodulatory effects on cellular and systemic level [53,54]. It was demonstrated that EVs from *H. polygyrus* suppress allergen-induced Type 2 innate responses and eosinophilia in mice [46] as well as macrophage activation [55]. EVs from *N. brasiliensis* were shown to protect mice against experimental colitis [28]. EVs from *F. hepatica* prevented experimental ulcerative colitis in mice by interfering with MAPK and NF-kB pathways and reducing pro-inflammatory cytokines, independently of T and B cells [59]. Additionally, EVs from *F. hepatica* were shown to induce mixed Th1/Th2 response in mice, lower the capacity of DCs to secrete TNF-α, but an enhance the expression of cell surface markers (CD80, CD86, CD40, OX40L, and SIGNR1) and elevation of SOCS1 and SOCS3, which can suppress IL-2 secretion from T cells in mice [60]. However not all EVs display beneficial immunomodulatory effects for potential application in the therapy of autoimmunity. Namely, EVs from *S. japonicum* increased the production of iNOS and TNF-α by macrophages and increased their expression of CD16/32, thus polarizing them towards M1 phenotype [61].

Our group was the first one who discovered that nematode *T. spiralis* also produces EVs [62]. Similar to other nematode's EVs, EVs found in ES L1 products of *T. spiralis* muscle larvae (TsEVs) are spherical, 30-80 nm in size, and of electron-lucent appearance under transmission electron microscopy (Fig. 2). Furthermore, it was found that TsEVs carry a selection of known ES L1 immunomodulatory proteins recognizable by 7C2C5 antibody which recognize 3 immunodominant epitopes in ES L1,

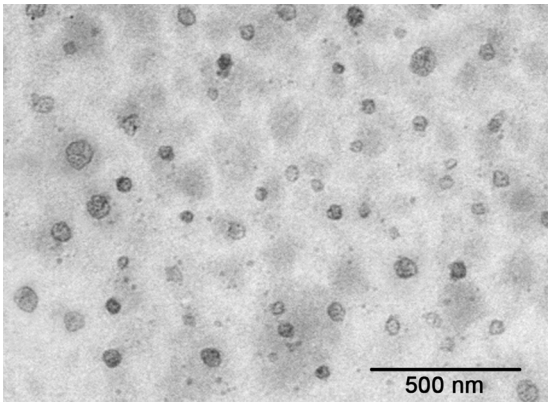

Fig. 2. Transmission electron microscopy image of extracellular vesicles isolated from *T. spiralis* ES L1 products (TsEVs)

two of which were detected in TsEVs. In analogy to EVs of several other helminths, which were found to be active immunomodulatory components of their ES products [54,63,64], we have shown that TsEVs are capable to exert some of the immunomodulatory effects by themselves. Namely, in cultures with human peripheral blood mononuclear cells (PBMC), TsEVs elevated IL-10 and decreased IL-17 production by PBMC, similarly to the effect of ES L1 [10,62]. This finding indicates that TsEVs are an active component of *T. spiralis* ES L1 and could be used for the design of a new therapeutic approach in fighting autoimmune diseases. Indeed, Yang et al. [37] have shown that *T. spiralis* derived EVs (TsEVs) have a great potential to mitigate TNBS-induced colitis by reducing Th1 and Th17 type of immune response and enhancing Th2 and regulatory arm of response. These findings suggest that in addition to soluble proteins in ES L1, TsEVs represent a new way by which *T. spiralis* can control host immune response, thereby revealing novel potential approaches that could be harnessed for the treatment of autoimmune diseases.

Another important aspect of the assessment of TsEVs as a potential therapeutic agent is the investigation of their composition i.e. determination of their active molecules. In general, EVs can interact with target cell in three major ways: 1. interaction of surface molecules on EVs and target cell leading to signal transduction; 2. phagocytosis of EVs by a target cell, leading to fusion of EVs with the membrane of early endosome and release of their content to the cytoplasm and 3. fusion of EVs with the plasma membrane and release of its cargo to the cytoplasm. While the interaction of surface molecules is often dependent on proteins, in two other types of interaction miRNAs are often found to be important active molecules. As for proteins as active molecules, we and others found known immunomodulatory proteins to be part of helminths' EVs [50,56,62,65]. However, it needs to be clarified whether particular proteins are able to induce the desirable immunomodulatory effects on their own, or if cooperation with other proteins/RNA is necessary. Studies also confirmed the presence of miRNA in helminths' EVs [44,66-68]. Those miRNAs are either similar to human miRNA with known immunomodulatory function, or possibly possess immune response-related targets, according to bioinformation analysis. Young et. al. [23] analyzed miRNA content of TsEVs and their bioinformatics analysis showed that some of these miRNA could regulate genes involved in immune response in humans. However, no experimental evidence was offered to date. Therefore, there is a need to identify/confirm specific proteins responsible for targeting immune cells as well as both protein and RNA effectors in order to better understand mechanisms by which TsEVs exert their immunomodulatory functions and ultimately to use them in novel therapeutic approaches to autoimmune diseases.

1.4. *Nanomedicine for autoimmune diseases*

EVs, as nano-sized communication systems capable of simultaneous transfer of multiple biomolecules, probably evolved as the most efficient way of targeted exchange of complex information necessary for efficient synchronization of numerous processes in multicellular organisms. In line with this, we witness the expansion of nanotechnology offering extraordinary possibilities of artificial nanoparticle or microparticle (NMP) use for the treatment of autoimmune diseases. There are several approaches for alleviating autoimmunity with NMPs [69,70] and all of these represent state-of-the-art knowledge on immune regulating mechanisms of immune response (Fig. 3).

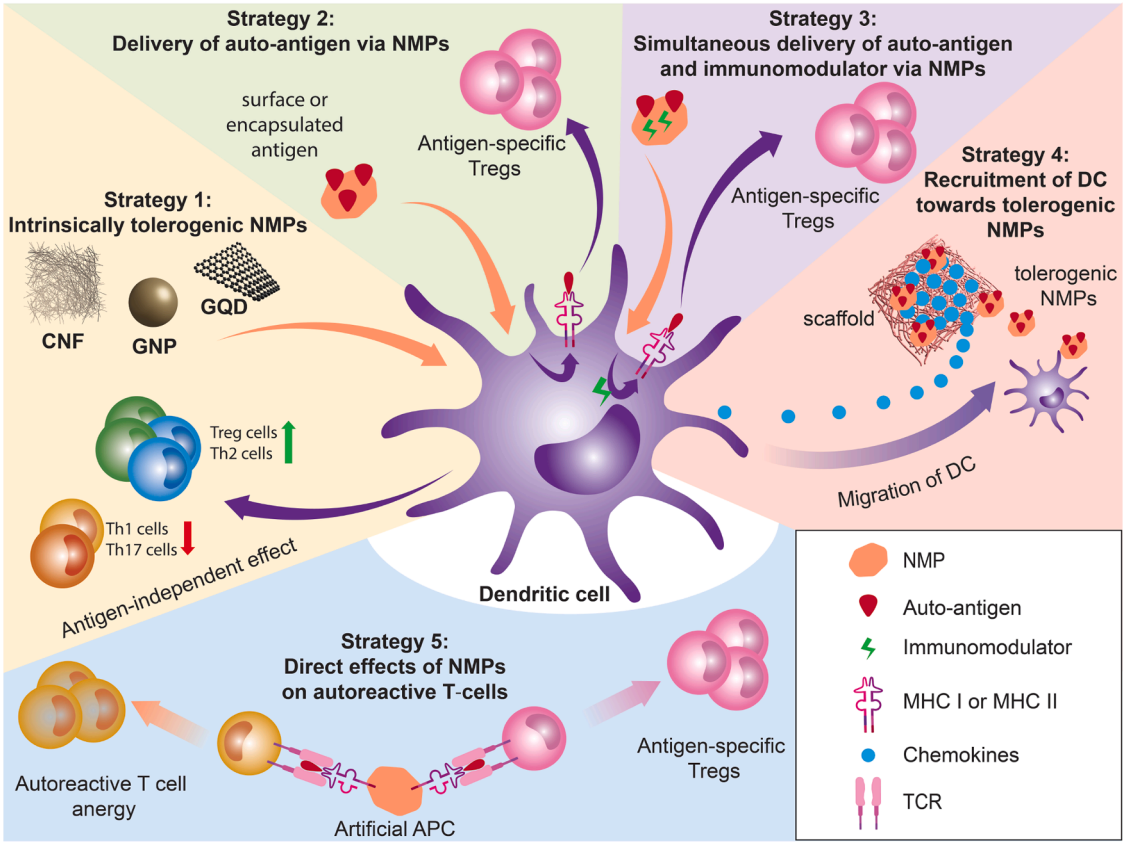

Fig. 3. Nanomedical approaches for restoring self-tolerance in autoimmunity. 1) Tolerogenic NMPs for the induction of tolerogenic DCs leading to antigen-independent modulation of T cell response towards Th2/Tregs, 2) Auto-antigen-conjugated NMPs for the induction of antigen-specific Tregs via DCs. 3) NMPs carrying both immunomodulatory agents and auto-antigen for the induction of antigen-specific Tregs via tolDC and, 4) Nanoscafolds, which release both DC attracting chemokines and tolerogenic NMPs, 5) Artificial APC - NMP carrying MHC-peptide complexes, for a direct induction of anergic and regulatory T cells (Design ST, Artwork MK).

The first approach includes the use of NMPs to deliver immunomodulatory molecules to DCs for the induction of tolDCs [70]. The methodology modulates broadly immune functions in an antigen-non-specific manner, and in this context, several studies reported successful amelioration or inhibition of EAE, by down-regulating the Th1 and Th17 responses, or by increasing the number of Treg cells [70]. This strategy was supported in the *in vitro* experiments [71], as well as *in vivo* in the EAE mice model [72-74]. Similar encouraging results have been published for T1D. In this regard, NPs delivering plasmid encoding mouse IL-10 [75], or anti-sense oligonucleotides for CD40, CD80, and CD86 were shown to block the activation of DCs and ameliorate the disease in mouse models of T1D [76]. Some NMPs display intrinsic immunomodulatory properties which could act via different mechanisms, such as the activation of scavenging receptors on monocytes and macrophages [77]. In this context, Allen et al. reported that MPs of low molecular weight PLGA posed an immunosuppressive effect on DCs, as judged by a reduced capacity for IL-12 secretion even after additional stimulation with lipopolysaccharide (LPS) [78]. Our studies identified several types of nanoparticles with intrinsic immunomodulatory properties, including spherical gold GNPs [79], cellulose nanofibers (CNF) [80–82], and graphene quantum dots (GQD) [83]. These NPs displayed inhibitory effects on TLR-induced phenotypic maturation of DCs, IL-12/IL-10 production ratio, and the polarization of Th1/Th17 response, but potentiating effects on the induction of Th2 response and regulatory T cells, although via different mechanisms [80,82-84]. It is especially important to emphasize that size, shape, and surface characteristics of NPs have significant effects on the interaction with DCs [85]. In line with this, smaller GNPs and GQDs (10-30nm in size) displayed stronger tolerogenic potential on DCs [79,83]. Both GNPs and GQDs were described as excellent photo-responsive delivery systems due to reactive surface chemistry [86,87], which could be easily harnessed to potentiate the immunomodulatory effects of specific *T. spiralis* components. In this context, the delivery of immunomodulatory agents via NPs displays better targeting effects on antigen-presenting cells and potentiates accumulation in inflammatory tissue due to enhanced permeability and retention (EPR) effect [88,89]. Moreover, the delivery of immunomodulatory molecules via NPs allows the potentiation of their effects due to accumulation and longer persistence in specific cellular compartments [90,91]. Besides NPs, nanofibrils are particularly attractive in delivering *T. spiralis* products due to the large surface available for modification, and slower biodistribution compared to spherical NPs. The surface of CNF is enriched with hydroxyl groups, so different components of ES L1 could be attached by using the same chemistry, thereby allowing their prolonged exposure in local tissues. Also, the induction of a specific anti-inflammatory response could be expected on infiltrating DCs, as we showed that CNF induces frustrated phagocytosis in DCs [80,82]. Besides, the surface of CNFs could be easily adjusted to potentiate a particular kind of regulatory T cell response, as we found that native CNF induces predominantly IDO-1-dependent induction of FoxP3+T cells, whereas ApA-CNF potentiated ILT-4/ILT-3-dependent induction of Tr-1 and suppressor CD8+ T cells [82]. However, the role of each of these regulatory T cell subsets in autoimmune diseases is yet to be defined.

The second approach for using NMPs in autoimmune diseases includes the delivery of autoantigen for presentation by DCs [70]. It is known that MPs with diameters of about 500 nm can serve as a surrogate to induce immune tolerance. In the absence of costimulatory signals, such MPs deliver autoantigenic epitopes to DCs without activating these cells [85]. The antigens are presented to autoreactive T cells and induce immune tolerance. Getts et al. reported that encephalitogenic myelin epitopes conjugated to carboxylated polystyrene MPs of 500 nm in diameter prevented the inflammatory cell infiltration into the CNS and induced T-cell tolerance in a relapse-remitting (RR) EAE mice model [77]. Similarly, PLGA-based NMPs carrying myelin antigen caused T-cell anergy, antigen-specific Tregs and ameliorated the ongoing RR EAE by reducing the infiltration of Th1 and Th17 cells, as well as inflammatory immune cells in the CNS [85,92]. Although ES L1 probably acts predominantly as an immunomodulatory agent via antigen-presenting cells, the finding that sera from multiple sclerosis patients cross-react with some ES L1 components suggests that ES L1 could potentially act as a mimicking antigen as well [93]. However, it is still unknown whether MPs conjugated with ES L1 could induce anergy in autoreactive T-cells from MS patients.

NMPs are attractive for other autoimmune diseases as well. Namely, the use of iron oxide NMPs coated with the islet-specific peptide glucose-6-phosphatase catalytic subunit-related protein (IGRP13-25)-MHCI or II reduced diabetes in NOD mice. Mice showed an increased number of type 1 regulatory (Tr1) cells expressing CD49b, LAG-3, ICOS, and TGF-β [94]. An interesting experiment showed that NPs loaded with a heat shock protein (65–6xP227), could prevent T1D when delivered orally to NOD mice [95]. The finding that ES L1 contains various immunomodulatory molecules, including proteinases and their inhibitors, heat shock proteins, kinases, phosphatases, glycosidases, endonucleases, DNA-binding proteins, and others [8], and engages different surface receptors on DCs [33,34], suggest that common mechanisms could be triggered by ES L1 delivered via NPs.

An alternative strategy, i.e. encapsulation of antigen, was developed to avoid the induction of antibody response to the surface-bound antigen [96]. In this context, encapsulation of recombinant human basic myelin protein (rhMBP) in poly ε caprolactone NPs reduced the disease score in MOG–induced EAE, when administrated subcutaneously, in a prophylactic manner [97]. Our results confirm that the delivery of NPs via degradable poly-ε caprolactone MPs enables quite efficient accumulation of the NPs to the infiltrating myeloid cells *in vivo* [98]. Intravenously delivered PLGA NMPs encapsulating BDC2.5 mimotope peptide prevented hyperglycemia for up to 50 days post transfer in NOD/SCID mice and the mechanisms included the development of intra-islet Foxp3+ Treg cells and increased expression of coinhibitory molecules PD-1 and CTLA-4 [99]. Besides PLGA NPs, PLGA nanofibers prepared by electrospinning technique represent an attractive delivery system. PLGA nanofibers enabled a precise release dynamic of a model drug, which could be controlled by thickening layers of PLGA nanofibers or via the inclusion of polycaprolactone around the PLGA nanofibers, thus allowing a delayed drug release onset [100]. Our preliminary results on ES L1 encapsulated into PLGA nanofibers prepared as subcutaneous implants, suggested that this system allows a slow release of ES L1 over longer periods. Moreover, ES L1-PLGA implants ameliorated symptoms in a rat model of EAE, unlike the equivalent doses of soluble ES-L1 and PLGA nanofiber implants themselves.

The third approach for using NMPs in autoimmunity is based on simultaneous delivery of both immunomodulators and antigens, to tolerize DCs which will then present the autoantigen in a tolerogenic form [70]. Examples of using tolerogenic molecules for delivery via NMPs include 2-(1H-indole-3-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) delivery that binds the aryl hydrocarbon receptor (AHR) on DCs and enhances their potential to trigger the differentiation of Foxp3+ Tregs [101]. DCs incubated with gold NPs loaded with ITE and myelin oligodendrocyte glycoprotein (MOG) peptide had tolerogenic properties and promoted FoxP3+ Treg differentiation [101]. Our findings on ESL1-induced IDO-1 expression on DCs and their increased capacity for the induction of FoxP3+ Tregs [33], also suggested that the AHR activation via the kynurenine pathway [102] is an important mechanism for tolerance induction. In line with this, NMPs carrying components of ES L1 could act in a combined way due to ES L1's both immunomodulatory [32] and autoantigenic properties [93]. One research group used PLGA nanoparticles carrying the complex of antigenic peptide together with rapamycin or rapamycin-loaded NPs co-administered with soluble antigen. Rapamycin was chosen because it induces regulatory DCs by inhibiting the mTOR pathway. *In vivo* experiments showed that SJL/J mice were protected from PLP- induced EAE, and that the relapse in mice with the established disease was inhibited [103]. Our finding that GQD by themselves could inhibit mTOR pathway and induce autophagy

in DCs, seems even better strategy for inducing a tolerogenic response in DCs [83] without using rapamycin. Generally, it is important to use appropriate NMP formulations that utilize antigen in combination with low doses of tolerogenic factors thus avoiding systemic immune suppression [70]. In this context, *T. spiralis* strategy for immune modulation is optimal since the infection does not compromise immune response to common pathogens [104] while protecting from the development of autoimmune diseases.

The fourth approach is based on the delivery of NMPs with the ability to increase DC recruitment to the injection site where autoantigen is taken up. Such cells are capable of trafficking to draining lymph nodes where they induce antigen-specific tolerance [70]. Cho et al. [105] delivered subcutaneously the combination of phagocytosable antigen-MPs together with the phagocytosable vitamin D3 MPs for the treatment of EAE. The MPs combination allowed intracellular delivery of antigen and vitamin D3 to induce tolerization of DCs. Additionally, when non-phagocytosable MPs coupled with GM-CSF and TGF-β were added simultaneously, they also allowed the recruitment of DCs and their precursors to the inflammation site (via GM-CSF) and simultaneous tolerization on the recruited DCs (via TGF-β) [105]. In line with this strategy is our finding that ES L1 upregulates CCR7 expression on DCs [33,34], which is involved in their migration towards lymph nodes, while still displaying tolDC properties [33,34,106]. However, it is still not clear which ES L1 components are responsible for the observed phenomenon.

The fifth approach is based on direct targeting or reprogramming of autoreactive T cells by NMPs [69]. In contrast to T-cell epitopes administrated in a soluble form leading to failure of tolerance induction and even severe impediments, the use of NPs has enabled the selectivity and efficiency of immune tolerance in comparison with current immunotherapies [69]. In this context, Otomo et al. [107] have used nanolipogels incorporated with an anti-CD4 mAb and a calcium/calmodulin-dependent protein kinase IV (CaMK4) inhibitor in order to inhibit CD4+ T cells. This NP complex inhibited the progression of EAE and suppressed the progression of lupus-like disease in MRL/lpr mice, possibly via blockade of Th17 differentiation, but without inducing general CD4+ T-cell depletion [107]. A more specific strategy includes the direct effects of nanomaterials against disease-relevant T-cells. An example is a use of NPs coated with T1D-relevant peptide–MHC class I with the capability to trigger the expansion of preexisting cognate memory-like low avidity regulatory CD8+ T cells. These cells were able to suppress diabetes in NOD mice by killing autoantigen-loaded professional APCs in the affected tissue(s) and draining lymph nodes [108]. The use of peptide-MHC class II coated NPs was shown to induce the differentiation of cognate effector/memory CD4+ T cells into Tr1-like cells with high expansion capabilities [109]. When these cells encounter costimulation-competent autoantigen-loaded APC expressing co-stimulatory molecules, they up-regulate the production of immunoregulatory cytokines, leading to the suppression of antigen presentation, autoreactive T-cell activation and recruitment, secretion of proinflammatory mediators, and formation of IL-10-producing Bregs [69,94]. The generation of artificial (a)APC is another example to directly modulate autoreactive T cells [85]. In this regard, disease-relevant peptide-MHC attached to aAPC (NP coated with anti-CD3 monoclonal antibody without anti-CD28 mAb) increased the adhesion with T cells and promoted the transition of autoreactive T cells into antigen-specific Tregs [94]. In contrast to soluble peptide-MHC complexes which trigger T-cell deletion, peptide-MHC complexes loaded onto aAPC expand memory-like autoregulatory T cells which suppressed the antigen-presenting ability of autoantigen-loaded APCs to T cells [108]. It has been shown that NPs coated with antigen-MHC-II molecules induced the expansion of antigen-specific Tr1 cells from T-cell precursor pools against multiple autoimmune diseases in mice, including T1D, EAE, and collagen-induced arthritis, without affecting systemic immunity [85,94]. These cells induce the production of regulatory cytokines which stimulate autoantigen-loaded APCs to present peptide-MHC complexes specifically to these Tr1-like cells and thus suppress the autoimmune response in a disease- and organ-specific manner.

2. Future perspectives

All of the reported studies regarding *in vivo* treatment with *T. spiralis* products implicated that the immunomodulatory capacity of these products and nanoparticles rely on the creation of an anti-inflammatory environment by the induction of Th2 and regulatory immune responses. Although some individual components of *T. spiralis* products were tested in the modulation of autoimmunity, future studies should shed more light on the immunomodulatory properties of different molecules from the composition of *T. spiralis* antigens that may be good candidates for the creation of recombinant molecules or artificial EVs with the potential to restrain the autoimmune diseases. The isolation of individual *T. spiralis* components would allow a better understanding of the underlining mechanisms involved in modulation of DCs, T cells, and other immune cells, their migration, antigen presentation, mimicking of autoantigen, as well as the possibility to recombine these components into an artificial system that would allow the complex multilevel control of the host immune response. The finding that *T. spiralis*, like other parasites, uses EVs to deliver information to the host immune system justifies the use of nanomedicines in the reconstruction of a nano delivery system that would enable precise control of immune response in humans. EVs in general have important properties that make them a suitable foundation for new therapeutic approaches: they carry complex bioinformation, pass blood-tissue barriers, they are targeted and can be altered through bioengineering. The biggest challenge in terms of using TsEVs as a therapeutic in the treatment of autoimmune diseases is their large-scale production from ES L1. This could be overcome by designing artificial/biomimetic EVs consisting of specific NMPs with targeting molecules on the surface and selected cargo of active protein/miRNA combination.

Another approach in using TsEVs for the development of novel therapy for autoimmune diseases would be to integrate them with NMPs. In that respect, three major directions should be explored: 1. Whether NPs and TsEVs, as two independent but simultaneous therapies, have synergistic effect; 2. Whether NPs interact with EVs during simultaneous therapy and if so, how does it reflects on their therapeutic efficacy; and 3. Could TsEVs be reengineered using NPs to be more efficient as therapeutics? However, the relationship between EVs and therapeutic NPs, in general, is a virtually unexplored field. There is almost no data on simultaneous EVs and NPs therapy. Few studies that tackle this field state that EVs might be a mechanism of disposal of NPs after their uptake by macrophages [110] and that positively charged iron oxide-based NPs can influence cells to increase the production of EVs [111]. As for how EVs can be engineered by NPs to increase their efficacy, there is somewhat more data. EVs can be protected by encapsulation with a nanofilm of supramolecular complexes of ferric ions (Fe^{3+}) and tannic acid which is controllably degraded [112]. Carbide quantum dots (V2C QDs) photothermal agents could be packed within EVs to increase the efficacy of its delivery to the nucleus [113]. Also, the therapeutic efficacy of gold NPs can be increased by using EVs for enhanced blood-brain barrier penetration [114]. Having in mind the considerable therapeutic potential of both ES L1 soluble proteins, EVs and NPs it will be remarkably interesting to see how this new field will evolve in directions of specific targeting, lower toxicity, and increased efficacy for the benefit of patients for whose diseases there is still no cure.

Cumulatively, *T. spiralis* display protective effects in autoimmunity via potentiation of Th2 and regulatory T cell response, predominantly, but not limited to, induction of stable tolerogenic DCs. The complex effects of *T. spiralis* are mediated by a vast number of products, including the extremely potent TsEVs, which carry multiple information packed in nanosized particles. Indeed, numerous independent data confirmed that

nanomedical approaches carrying complex information for the induction of tolerance are the most promising for the treatment of autoimmune diseases. Although a long way is ahead of us before we could completely replicate natural nano-delivery systems build through evolution, a clear path is being opened from a careful examination of the parasite-host interactions.

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References

[1] S. Jörg, D.A. Grohme, M. Erzler, M. Binsfeld, A. Haghikia, D.N. Müller, R. A. Linker, M. Kleinewietfeld, Environmental factors in autoimmune diseases and their role in multiple sclerosis, Cell. Mol. Life Sci. 73 (2016) 4611–4622, https://doi.org/10.1007/s00018-016-2311-1.

[2] T.B. Smallwood, P.R. Giacomin, A. Loukas, J.P. Mulvenna, R.J. Clark, J.J. Miles, Helminth Immunomodulation in Autoimmune Disease, Front. Immunol. 8 (2017), https://doi.org/10.3389/fimmu.2017.00453.

[3] R.M. Maizels, H.J. McSorley, D.J. Smyth, Helminths in the hygiene hypothesis: sooner or later? Clinical & Experimental Immunology 177 (2014) 38–46, https://doi.org/10.1111/cei.12353.

[4] J.-F. Bach, The hygiene hypothesis in autoimmunity: the role of pathogens and commensals, Nat. Rev. Immunol. 18 (2018) 105–120, https://doi.org/10.1038/nri.2017.111.

[5] J. Sotillo, I. Ferreira, J. Potriquet, T. Laha, S. Navarro, A. Loukas, J. Mulvenna, Changes in protein expression after treatment with Ancylostoma caninum excretory/secretory products in a mouse model of colitis, Sci. Rep. 7 (2017) 41883, https://doi.org/10.1038/srep41883.

[6] Y.A. Khan, S. Umar, S.M.A. Abidi, Somatic Antigens of Tropical Liver Flukes Ameliorate Collagen-Induced Arthritis in Wistar Rats, PLoS One 10 (2015), e0126429, https://doi.org/10.1371/journal.pone.0126429.

[7] J. Correale, M. Farez, Helminth Antigens Modulate Immune Responses in Cells from Multiple Sclerosis Patients through TLR2-Dependent Mechanisms, J. Immunol. 183 (2009) 5999–6012, https://doi.org/10.4049/jimmunol.0900897.

[8] J. Ding, X. Liu, X. Bai, Y. Wang, J. Li, C. Wang, S. Li, M. Liu, X. Wang, Trichinella spiralis: inflammation modulator, J. Helminthol. 94 (2020), https://doi.org/10.1017/S0022149×20000802.

[9] Z. Wu, I. Nagano, Y. Takahashi, Trichinella: What is going on during nurse cell formation? Vet. Parasitol. 194 (2013) 155–159, https://doi.org/10.1016/j.vetpar.2013.01.044.

[10] L. Sofronic-Milosavljevic, N. Ilic, E. Pinelli, A. Gruden-Movsesijan, Secretory Products of Trichinella spiralis Muscle Larvae and Immunomodulation: Implication for Autoimmune Diseases, Allergies, and Malignancies, Journal of Immunology Research (2015), e523875, https://doi.org/10.1155/2015/523875, 2015.

[11] Y. Zhao, M.Y. Liu, X.L. Wang, X.L. Liu, Y. Yang, H.B. Zou, S.M. Sun, L. Yu, B. Rosenthal, H.N. Shi, P. Boireau, X.P. Wu, Modulation of inflammatory bowel disease in a mouse model following infection with Trichinella spiralis, Vet. Parasitol. 194 (2013) 211–216, https://doi.org/10.1016/j.vetpar.2013.01.058.

[12] W. Zheng, Z. Ma, X. Sun, Y. Huang, B. Lu, X. Chen, X. Xue, X. Yang, X. Wu, Exposure time determines the protective effect of Trichinella spiralis on experimental colitis, Microb. Pathog. 147 (2020), https://doi.org/10.1016/j.micpath.2020.104263, 104263.

[13] A. Gruden-Movsesijan, N. Ilic, M. Mostarica-Stojkovic, S. Stosic-Grujicic, M. Milic, Lj. Sofronic-Milosavljevic, Trichinella spiralis: Modulation of experimental autoimmune encephalomyelitis in DA rats, Exp. Parasitol. 118 (2008) 641–647, https://doi.org/10.1016/j.exppara.2007.12.003.

[14] A. Gruden-Movsesijan, N. Ilic, M. Mostarica-Stojkovic, S. Stosic-Grujicic, M. Milic, L. Sofronic-Milosavljevic, Mechanisms of modulation of experimental autoimmune encephalomyelitis by chronic Trichinella spiralis infection in Dark Agouti rats, Parasite Immunol. 32 (2010) 450–459, https://doi.org/10.1111/j.1365-3024.2010.01207.x.

[15] C. Aranzamendi, L. Sofronic-Milosavljevic, E. Pinelli, Helminths: Immunoregulation and Inflammatory Diseases—Which Side Are Trichinella spp. and Toxocara spp. on? Journal of Parasitology Research (2013) https://doi.org/10.1155/2013/329438, 2013e329438.

[16] Y. Cheng, X. Zhu, X. Wang, Q. Zhuang, X. Huyan, X. Sun, J. Huang, B. Zhan, X. Zhu, Trichinella spiralis Infection Mitigates Collagen-Induced Arthritis via Programmed Death 1-Mediated Immunomodulation, Front. Immunol. 9 (2018), https://doi.org/10.3389/fimmu.2018.01566.

[17] Y. Osada, K. Morita, S. Tahara, T. Ishihara, Z. Wu, I. Nagano, Y. Maekawa, S. Nakae, K. Sudo, T. Kanazawa, Th2 signals are not essential for the anti-arthritic effects of Trichinella spiralis in mice, Parasite Immunol. 42 (2020), https://doi.org/10.1111/pim.12677 e12677.

[18] K.A. Saunders, T. Raine, A. Cooke, C.E. Lawrence, Inhibition of Autoimmune Type 1 Diabetes by Gastrointestinal Helminth Infection, Infect. Immun. 75 (2007) 397–407, https://doi.org/10.1128/IAI.00664-06.

[19] M.W. Robinson, B. Connolly, Proteomic analysis of the excretory-secretory proteins of the Trichinella spiralis L1 larva, a nematode parasite of skeletal muscle, Proteomics 5 (2005) 4525–4532, https://doi.org/10.1002/pmic.200402057.

[20] N. Ilić, A. Gruden-Movsesijan, M. Živojinović, L. Sofronić-Milosavljević, Characteristic band pattern in western blots for specific detection of anti-Trichinella spiralis antibodies in different host species, Acta Vet. (Beogr) 64 (2014) 33–43, https://doi.org/10.2478/acve-2014-0004.

[21] J. Cvetkovic, Lj. Sofronic-Milosavljevic, N. Ilic, M. Gnjatovic, I. Nagano, A. Gruden-Movsesijan, Immunomodulatory potential of particular Trichinella spiralis muscle larvae excretory–secretory components, Int. J. Parasitol. 46 (2016) 833–842, https://doi.org/10.1016/j.ijpara.2016.07.008.

[22] Y. Wang, X. Bai, H. Zhu, X. Wang, H. Shi, B. Tang, P. Boireau, X. Cai, X. Luo, M. Liu, X. Liu, Immunoproteomic analysis of the excretory-secretory products of Trichinella pseudospiralis adult worms and newborn larvae, Parasites & Vectors 10 (2017) 579, https://doi.org/10.1186/s13071-017-2522-9.

[23] X. Yang, Y. Yang, Y. Wang, B. Zhan, Y. Gu, Y. Cheng, X. Zhu, Excretory/Secretory Products from Trichinella spiralis Adult Worms Ameliorate DSS-Induced Colitis in Mice, PLoS One 9 (2014) e96454, https://doi.org/10.1371/journal.pone.0096454.

[24] S.A. Kang, M.-K. Park, S.K. Park, J.H. Choi, D.I. Lee, S.M. Song, H.S. Yu, Adoptive transfer of Trichinella spiralis-activated macrophages can ameliorate both Th1- and Th2-activated inflammation in murine models, Sci. Rep. 9 (2019) 6547, https://doi.org/10.1038/s41598-019-43057-1.

[25] Y. Motomura, H. Wang, Y. Deng, R.T. El-Sharkawy, E.F. Verdu, W.I. Khan, Helminth antigen-based strategy to ameliorate inflammation in an experimental model of colitis, Clinical & Experimental Immunology 155 (2009) 88–95, https://doi.org/10.1111/j.1365-2249.2008.03805.x.

[26] J. Xu, M. Liu, P. Yu, L. Wu, Y. Lu, Effect of recombinant Trichinella spiralis cysteine proteinase inhibitor on TNBS-induced experimental inflammatory bowel disease in mice, Int. Immunopharmacol. 66 (2019) 28–40, https://doi.org/10.1016/j.intimp.2018.10.043.

[27] J. Xu, L. Wu, P. Yu, M. Liu, Y. Lu, Effect of two recombinant Trichinella spiralis serine protease inhibitors on TNBS-induced experimental colitis of mice, Clinical & Experimental Immunology 194 (2018) 400–413, https://doi.org/10.1111/cei.13199.

[28] J. Pang, J. Ding, L. Zhang, Y. Zhang, Y. Yang, X. Bai, X. Liu, X. Jin, H. Guo, Y. Yang, M. Liu, Effect of recombinant serine protease from adult stage of Trichinella spiralis on TNBS-induced experimental colitis in mice, Int. Immunopharmacol. 86 (2020), 106699, https://doi.org/10.1016/j.intimp.2020.106699.

[29] L. Du, H. Tang, Z. Ma, J. Xu, W. Gao, J. Chen, W. Gan, Z. Zhang, X. Yu, X. Zhou, X. Hu, The Protective Effect of the Recombinant 53-kDa Protein of Trichinella spiralis on Experimental Colitis in Mice, Dig Dis Sci 56 (2011) 2810–2817, https://doi.org/10.1007/s10620-011-1689-8.

[30] L.M. Kuijk, E.J. Klaver, G. Kooij, S.M.A. van der Pol, P. Heijnen, S.C.M. Bruijns, H. Kringel, E. Pinelli, G. Kraal, H.E. de Vries, C.D. Dijkstra, G. Bouma, I. van Die, Soluble helminth products suppress clinical signs in murine experimental autoimmune encephalomyelitis and differentially modulate human dendritic cell activation, Mol. Immunol. 51 (2012) 210–218, https://doi.org/10.1016/j.molimm.2012.03.020.

[31] Lj. Sofronic-Milosavljevic, I. Radovic, N. Ilic, I. Majstorovic, J. Cvetkovic, A. Gruden-Movsesijan, Application of dendritic cells stimulated with Trichinella spiralis excretory–secretory antigens alleviates experimental autoimmune encephalomyelitis, Med Microbiol Immunol 202 (2013) 239–249, https://doi.org/10.1007/s00430-012-0286-6.

[32] I. Radovic, A. Gruden-Movsesijan, N. Ilic, J. Cvetkovic, S. Mojsilovic, M. Devic, L. Sofronic-Milosavljevic, Immunomodulatory effects of Trichinella spiralis-derived excretory–secretory antigens, Immunol Res 61 (2015) 312–325, https://doi.org/10.1007/s12026-015-8626-4.

[33] N. Ilic, A. Gruden-Movsesijan, J. Cvetkovic, S. Tomic, D.B. Vucevic, C. Aranzamendi, M. Colic, E. Pinelli, L. Sofronic-Milosavljevic, Trichinella spiralis Excretory–Secretory Products Induce Tolerogenic Properties in Human Dendritic Cells via Toll-Like Receptors 2 and 4, Front. Immunol. 9 (2018), https://doi.org/10.3389/fimmu.2018.00011.

[34] J. Cvetkovic, N. Ilic, A. Gruden-Movsesijan, S. Tomic, N. Mitic, E. Pinelli, L. Sofronic-Milosavljevic, DC-SIGN signalling induced by Trichinella spiralis products contributes to the tolerogenic signatures of human dendritic cells, Sci. Rep. 10 (2020) 1–14.

[35] A. Marcilla, L. Martin-Jaular, M. Trelis, A. de Menezes-Neto, A. Osuna, D. Bernal, C. Fernandez-Becerra, I.C. Almeida, H.A. del Portillo, Extracellular vesicles in parasitic diseases, Journal of Extracellular Vesicles 3 (2014) 25040, https://doi.org/10.3402/jev.v3.25040.

[36] M. Yáñez-Mó, P.R.-M. Siljander, Z. Andreu, A.B. Zavec, F.E. Borràs, E.I. Buzas, K. Buzas, E. Casal, F. Cappello, J. Carvalho, E. Colás, A.C. Silva, S. Fais, J. M. Falcon-Perez, I.M. Ghobrial, B. Giebel, M. Gimona, M. Graner, I. Gursel, M. Gursel, N.H.H. Heegaard, A. Hendrix, P. Kierulf, K. Kokubun, M. Kosanovic, V. Kralj-Iglic, E.-M. Krämer-Albers, S. Laitinen, C. Lässer, T. Lener, E. Ligeti, A. Linē, G. Lipps, A. Llorente, J. Lötvall, M. Manček-Keber, A. Marcilla, M. Mittelbrunn, I. Nazarenko, E.N.M.N.-'t Hoen, T.A. Nyman, L. O'Driscoll, M. Olivan, C. Oliveira, É. Pállinger, H.A. del Portillo, J. Reventós, M. Rigau, E. Rohde, M. Sammar, F. Sánchez-Madrid, N. Santarém, K. Schallmoser, M. S. Ostenfeld, W. Stoorvogel, R. Stukelj, S.G.V. der Grein, M.H. Vasconcelos, M.H.

M. Wauben, O.D. Wever, Biological properties of extracellular vesicles and their physiological functions, Journal of Extracellular Vesicles 4 (2015) 27066, https://doi.org/10.3402/jev.v4.27066.

[37] C. Théry, K.W. Witwer, E. Aikawa, M.J. Alcaraz, J.D. Anderson, R. Andriantsitohaina, A. Antoniou, T. Arab, F. Archer, G.K. Atkin-Smith, D. C. Ayre, J.-M. Bach, D. Bachurski, H. Baharvand, L. Balaj, S. Baldacchino, N. N. Bauer, A.A. Baxter, M. Bebawy, C. Beckham, A.B. Zavec, A. Benmoussa, A. C. Berardi, P. Bergese, E. Bielska, C. Blenkiron, S. Bobis-Wozowicz, E. Boilard, W. Boireau, A. Bongiovanni, F.E. Borràs, S. Bosch, C.M. Boulanger, X. Breakefield, A.M. Breglio, M.Á. Brennan, D.R. Brigstock, A. Brisson, M.L. Broekman, J. F. Bromberg, P. Bryl-Górecka, S. Buch, A.H. Buck, D. Burger, S. Busatto, D. Buschmann, B. Bussolati, E.I. Buzás, J.B. Byrd, G. Camussi, D.R. Carter, S. Caruso, L.W. Chamley, Y.-T. Chang, C. Chen, S. Chen, L. Cheng, A.R. Chin, A. Clayton, S.P. Clerici, A. Cocks, E. Cocucci, R.J. Coffey, A. Cordeiro-da-Silva, Y. Couch, F.A. Coumans, B. Coyle, R. Crescitelli, M.F. Criado, C. D'Souza-Schorey, S. Das, A.D. Chaudhuri, P. de Candia, E.F.D.S. Junior, O.D. Wever, H.A. del Portillo, T. Demaret, S. Deville, A. Devitt, B. Dhondt, D.D. Vizio, L.C. Dieterich, V. Dolo, A.P.D. Rubio, M. Dominici, M.R. Dourado, T.A. Driedonks, F.V. Duarte, H.M. Duncan, R.M. Eichenberger, K. Ekström, S.E. Andaloussi, C. Elie-Caille, U. Erdbrügger, J.M. Falcón-Pérez, F. Fatima, J.E. Fish, M. Flores-Bellver, A. Försönits, A. Frelet-Barrand, F. Fricke, G. Fuhrmann, S. Gabrielsson, A. Gámez-Valero, C. Gardiner, K. Gärtner, R. Gaudin, Y.S. Gho, B. Giebel, C. Gilbert, M. Gimona, I. Giusti, D.C. Goberdhan, A. Görgens, S.M. Gorski, D.W. Greening, J. C. Gross, A. Gualerzi, G.N. Gupta, D. Gustafson, A. Handberg, R.A. Haraszti, P. Harrison, H. Hegyesi, A. Hendrix, A.F. Hill, F.H. Hochberg, K.F. Hoffmann, B. Holder, H. Holthofer, B. Hosseinkhani, G. Hu, Y. Huang, V. Huber, S. Hunt, A. G.-E. Ibrahim, T. Ikezu, J.M. Inal, M. Isin, A. Ivanova, H.K. Jackson, S. Jacobsen, S.M. Jay, M. Jayachandran, G. Jenster, L. Jiang, S.M. Johnson, J.C. Jones, A. Jong, T. Jovanovic-Talisman, S. Jung, R. Kalluri, S. Kano, S. Kaur, Y. Kawamura, E.T. Keller, D. Khamari, E. Khomyakova, A. Khvorova, P. Kierulf, K.P. Kim, T. Kislinger, M. Klingeborn, D.J.K.II M. Kornek, M.M. Kosanović, Á. F. Kovács, E.-M. Krämer-Albers, S. Krasemann, M. Krause, I.V. Kurochkin, G. D. Kusuma, S. Kuypers, S. Laitinen, S.M. Langevin, L.R. Languino, J. Lannigan, C. Lässer, L.C. Laurent, G. Lavieu, E. Lázaro-Ibáñez, S.L. Lay, M.-S. Lee, Y.X.F. Lee, D.S. Lemos, M. Lenassi, A. Leszczynska, I.T. Li, K. Liao, S.F. Libregts, E. Ligeti, R. Lim, S.K. Lim, A. Linē, K. Linnemannstöns, A. Llorente, C.A. Lombard, M. J. Lorenowicz, Á.M. Lörincz, J. Lötvall, J. Lovett, M.C. Lowry, X. Loyer, Q. Lu, B. Lukomska, T.R. Lunavat, S.L. Maas, H. Malhi, A. Marcilla, J. Mariani, J. Mariscal, E.S. Martens-Uzunova, L. Martin-Jaular, M.C. Martinez, V.R. Martins, M. Mathieu, S. Mathivanan, M. Maugeri, L.K. McGinnis, M.J. McVey, D.G.M. Jr, K.L. Meehan, I. Mertens, V.R. Minciacchi, A. Möller, M.M. Jørgensen, A. Morales-Kastresana, J. Morhayim, F. Mullier, M. Muraca, L. Musante, V. Mussack, D. C. Muth, K.H. Myburgh, T. Najrana, M. Nawaz, I. Nazarenko, P. Nejsum, C. Neri, T. Neri, R. Nieuwland, L. Nimrichter, J.P. Nolan, E.N.N.-'t Hoen, N.N. Hooten, L. O'Driscoll, T. O'Grady, A. O'Loghlen, T. Ochiya, M. Olivier, A. Ortiz, L.A. Ortiz, X. Osteikoetxea, O. Østergaard, M. Ostrowski, J. Park, D.M. Pegtel, H. Peinado, F. Perut, M.W. Pfaffl, D.G. Phinney, B.C. Pieters, R.C. Pink, D.S. Pisetsky, E.P. von Strandmann, I. Polakovicova, I.K. Poon, B.H. Powell, I. Prada, L. Pulliam, P. Quesenberry, A. Radeghieri, R.L. Raffai, S. Raimondo, J. Rak, M.I. Ramirez, G. Raposo, M.S. Rayyan, N. Regev-Rudzki, F.L. Ricklefs, P.D. Robbins, D. D. Roberts, S.C. Rodrigues, E. Rohde, S. Rome, K.M. Rouschop, A. Rughetti, A. E. Russell, P. Saá, S. Sahoo, E. Salas-Huenuleo, C. Sánchez, J.A. Saugstad, M. J. Saul, R.M. Schiffelers, R. Schneider, T.H. Schøyen, A. Scott, E. Shahaj, S. Sharma, O. Shatnyeva, F. Shekari, G.V. Shelke, A.K. Shetty, K. Shiba, P.R.-M. Siljander, A.M. Silva, A. Skowronek, O.L.S. II, R.P. Soares, B.W. Sódar, C. Soekmadji, J. Sotillo, P.D. Stahl, W. Stoorvogel, S.L. Stott, E.F. Strasser, S. Swift, H. Tahara, M. Tewari, K. Timms, S. Tiwari, R. Tixeira, M. Tkach, W. S. Toh, R. Tomasini, A.C. Torrecilhas, J.P. Tosar, V. Toxavidis, L. Urbanelli, P. Vader, B.W. van Balkom, S.G. van der Grein, J.V. Deun, M.J. van Herwijnen, K. V. Keuren-Jensen, G. van Niel, M.E. van Royen, A.J. van Wijnen, M. H. Vasconcelos, I.J.V. Jr, T.D. Veit, L.J. Vella, É. Velot, F.J. Verweij, B. Vestad, J. L. Viñas, T. Visnovitz, K.V. Vukman, J. Wahlgren, D.C. Watson, M.H. Wauben, A. Weaver, J.P. Webber, V. Weber, A.M. Wehman, D.J. Weiss, J.A. Welsh, S. Wendt, A.M. Wheelock, Z. Wiener, L. Witte, J. Wolfram, A. Xagorari, P. Xander, J. Xu, X. Yan, M. Yáñez-Mó, H. Yin, Y. Yuana, V. Zappulli, J. Zarubova, V. Žėkas, J. Zhang, Z. Zhao, L. Zheng, A.R. Zheutlin, A.M. Zickler, P. Zimmermann, A. M. Zivkovic, D. Zocco, E.K. Zuba-Surma, Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines, Journal of Extracellular Vesicles 7 (2018), 1535750, https://doi.org/10.1080/20013078.2018.1535750.

[38] A. Marcilla, M. Trelis, A. Cortés, J. Sotillo, F. Cantalapiedra, M.T. Minguez, M. L. Valero, M.M.S. del Pino, C. Muñoz-Antoli, R. Toledo, D. Bernal, Extracellular Vesicles from Parasitic Helminths Contain Specific Excretory/Secretory Proteins and Are Internalized in Intestinal Host Cells, PLoS One 7 (2012) e45974, https://doi.org/10.1371/journal.pone.0045974.

[39] D.W. Kifle, J. Sotillo, M.S. Pearson, A. Loukas, Extracellular vesicles as a target for the development of anti-helminth vaccines, Emerging Topics in Life Sciences. 1 (2017) 659–665. https://doi.org/10.1042/ETLS20170095.

[40] K. Andresen, P.E. Simonsen, B.J. Andersen, A. Birch-Andersen, Echinostoma caproni in mice: Shedding of antigens from the surface of an intestinal trematode, Int. J. Parasitol. 19 (1989) 111–118, https://doi.org/10.1016/0020-7519(89)90028-3.

[41] J. Sotillo, M. Pearson, J. Potriquet, L. Becker, D. Pickering, J. Mulvenna, A. Loukas, Extracellular vesicles secreted by Schistosoma mansoni contain protein vaccine candidates, Int. J. Parasitol. 46 (2016) 1–5, https://doi.org/10.1016/j.ijpara.2015.09.002.

[42] L. Zhu, J. Liu, J. Dao, K. Lu, H. Li, H. Gu, J. Liu, X. Feng, G. Cheng, Molecular characterization of S. japonicum exosome-like vesicles reveals their regulatory roles in parasite-host interactions, Sci. Rep. 6 (2016) 25885, https://doi.org/10.1038/srep25885.

[43] S. Chaiyadet, J. Sotillo, M. Smout, C. Cantacessi, M.K. Jones, M.S. Johnson, L. Turnbull, C.B. Whitchurch, J. Potriquet, M. Laohaviroj, J. Mulvenna, P. J. Brindley, J.M. Bethony, T. Laha, B. Sripa, A. Loukas, Carcinogenic Liver Fluke Secretes Extracellular Vesicles That Promote Cholangiocytes to Adopt a Tumorigenic Phenotype, J. Infect. Dis. 212 (2015) 1636–1645, https://doi.org/10.1093/infdis/jiv291.

[44] D. Bernal, M. Trelis, S. Montaner, F. Cantalapiedra, A. Galiano, M. Hackenberg, A. Marcilla, Surface analysis of Dicrocoelium dendriticum. The molecular characterization of exosomes reveals the presence of miRNAs, J. Proteomics 105 (2014) 232–241, https://doi.org/10.1016/j.jprot.2014.02.012.

[45] G.B. dos Santos, K.M. Monteiro, E.D. da Silva, M.E. Battistella, H.B. Ferreira, A. Zaha, Excretory/secretory products in the Echinococcus granulosus metacestode: is the intermediate host complacent with infection caused by the larval form of the parasite? Int. J. Parasitol. 46 (2016) 843–856, https://doi.org/10.1016/j.ijpara.2016.07.009.

[46] A.H. Buck, G. Coakley, F. Simbari, H.J. McSorley, J.F. Quintana, T.Le Bihan, S. Kumar, C. Abreu-Goodger, M. Lear, Y. Harcus, A. Ceroni, S.A. Babayan, M. Blaxter, A. Ivens, R.M. Maizels, Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity, Nat. Commun. 5 (2014), https://doi.org/10.1038/ncomms6488, 5488.

[47] E.P. Hansen, H. Kringel, A.R. Williams, P. Nejsum, Secretion of RNA-Containing Extracellular Vesicles by the Porcine Whipworm, Trichuris suis, Para 101 (2015) 336–340, https://doi.org/10.1645/14-714.1.

[48] T. Tzelos, J.B. Matthews, A.H. Buck, F. Simbari, D. Frew, N.F. Inglis, K. McLean, A.J. Nisbet, C.B.A. Whitelaw, D.P. Knox, T.N. McNeilly, A preliminary proteomic characterisation of extracellular vesicles released by the ovine parasitic nematode, Teladorsagia circumcincta, Vet. Parasitol. 221 (2016) 84–92, https://doi.org/10.1016/j.vetpar.2016.03.008.

[49] H.Y. Gu, N.D. Marks, A.D. Winter, W. Weir, T. Tzelos, T.N. McNeilly, C. Britton, E. Devaney, Conservation of a microRNA cluster in parasitic nematodes and profiling of miRNAs in excretory-secretory products and microvesicles of Haemonchus contortus, PLoS Negl.Trop. Dis. 11 (2017), https://doi.org/10.1371/journal.pntd.0006056 e0006056.

[50] M. Zamanian, L.M. Fraser, P.N. Agbedanu, H. Harischandra, A.R. Moorhead, T. A. Day, L.C. Bartholomay, M.J. Kimber, Release of Small RNA-containing Exosome-like Vesicles from the Human Filarial Parasite Brugia malayi, PLoS Negl. Trop. Dis. 9 (2015), https://doi.org/10.1371/journal.pntd.0004069 e0004069.

[51] E.P. Hansen, B. Fromm, S.D. Andersen, A. Marcilla, K.L. Andersen, A. Borup, A. R. Williams, A.R. Jex, R.B. Gasser, N.D. Young, R.S. Hall, A. Stensballe, V. Ovchinnikov, Y. Yan, M. Fredholm, S.M. Thamsborg, P. Nejsum, Exploration of extracellular vesicles from Ascaris suum provides evidence of parasite–host cross talk, Journal of Extracellular Vesicles 8 (2019), 1578116, https://doi.org/10.1080/20013078.2019.1578116.

[52] L. Tritten, T.G. Geary, Helminth extracellular vesicles in host–parasite interactions, Curr. Opin. Microbiol. 46 (2018) 73–79, https://doi.org/10.1016/j.mib.2018.08.002.

[53] A. Zakeri, E.P. Hansen, S.D. Andersen, A.R. Williams, P. Nejsum, Immunomodulation by Helminths: Intracellular Pathways and Extracellular Vesicles, Front. Immunol 9 (2018), https://doi.org/10.3389/fimmu.2018.02349.

[54] R.M. Eichenberger, M.H. Talukder, M.A. Field, P. Wangchuk, P. Giacomin, A. Loukas, J. Sotillo, Characterization of Trichuris muris secreted proteins and extracellular vesicles provides new insights into host–parasite communication, Journal of Extracellular Vesicles 7 (2018), 1428004, https://doi.org/10.1080/20013078.2018.1428004.

[55] G. Coakley, J.L. McCaskill, J.G. Borger, F. Simbari, E. Robertson, M. Millar, Y. Harcus, H.J. McSorley, R.M. Maizels, A.H. Buck, Extracellular Vesicles from a Helminth Parasite Suppress Macrophage Activation and Constitute an Effective Vaccine for Protective Immunity, Cell Rep. 19 (2017) 1545–1557, https://doi.org/10.1016/j.celrep.2017.05.001.

[56] M.C. Nicolao, C.R. Rodrigues, A.C. Cumino, Extracellular vesicles from Echinococcus granulosus larval stage: Isolation, characterization and uptake by dendritic cells, PLoS Negl.Trop. Dis. 13 (2019), https://doi.org/10.1371/journal.pntd.0007032 e0007032.

[57] S. Chaiyadet, J. Sotillo, W. Krueajampa, S. Thongsen, P.J. Brindley, B. Sripa, A. Loukas, T. Laha, Vaccination of hamsters with Opisthorchis viverrini extracellular vesicles and vesicle-derived recombinant tetraspanins induces antibodies that block vesicle uptake by cholangiocytes and reduce parasite burden after challenge infection, PLoS Negl.Trop. Dis. 13 (2019), https://doi.org/10.1371/journal.pntd.0007450 e0007450.

[58] M.E. Kuipers, E.N.M.N.-'t Hoen, A.J. van der Ham, A. Ozir-Fazalalikhan, D. L. Nguyen, C.M. de Korne, R.I. Koning, J.J. Tomes, K.F. Hoffmann, H.H. Smits, C. H. Hokke, DC-SIGN mediated internalisation of glycosylated extracellular vesicles from Schistosoma mansoni increases activation of monocyte-derived dendritic cells, Journal of Extracellular Vesicles 9 (2020), 1753420, https://doi.org/10.1080/20013078.2020.1753420.

[59] J. Roig, M.L. Saiz, A. Galiano, M. Trelis, F. Cantalapiedra, C. Monteagudo, E. Giner, R.M. Giner, M.C. Recio, D. Bernal, F. Sánchez-Madrid, A. Marcilla, Extracellular Vesicles From the Helminth Fasciola hepatica Prevent DSS-Induced Acute Ulcerative Colitis in a T-Lymphocyte Independent Mode, Front. Microbiol. 9 (2018), https://doi.org/10.3389/fmicb.2018.01036.

[60] A. Murphy, K. Cwiklinski, R. Lalor, B. O'Connell, M.W. Robinson, J. Gerlach, L. Joshi, M. Kilcoyne, J.P. Dalton, S.M. O'Neill, Fasciola hepatica Extracellular Vesicles isolated from excretory-secretory products using a gravity flow method modulate dendritic cell phenotype and activity, PLoS Negl.Trop. Dis. 14 (2020), https://doi.org/10.1371/journal.pntd.0008626 e0008626.
[61] L. Wang, Z. Li, J. Shen, Z. Liu, J. Liang, X. Wu, X. Sun, Z. Wu, Exosome-like vesicles derived by Schistosoma japonicum adult worms mediates M1 type immune- activity of macrophage, Parasitol Res 114 (2015) 1865–1873, https://doi.org/10.1007/s00436-015-4373-7.
[62] M. Kosanović, J. Cvetković, A. Gruden-Movsesijan, S. Vasilev, M. Svetlana, N. Ilić, L. Sofronić-Milosavljević, Trichinella spiralis muscle larvae release extracellular vesicles with immunomodulatory properties, Parasite Immunol. 41 (2019) e12665, https://doi.org/10.1111/pim.12665.
[63] H. Harischandra, W. Yuan, H.J. Loghry, M. Zamanian, M.J. Kimber, Profiling extracellular vesicle release by the filarial nematode Brugia malayi reveals sex-specific differences in cargo and a sensitivity to ivermectin, PLoS Negl.Trop. Dis. 12 (2018), https://doi.org/10.1371/journal.pntd.0006438 e0006438.
[64] M. Siles-Lucas, C. Sánchez-Ovejero, M. González-Sánchez, E. González, J. M. Falcón-Pérez, B. Boufana, F. Fratini, A. Casulli, R. Manzano-Román, Isolation and characterization of exosomes derived from fertile sheep hydatid cysts, Vet. Parasitol. 236 (2017) 22–33, https://doi.org/10.1016/j.vetpar.2017.01.022.
[65] F. Simbari, J. McCaskill, G. Coakley, M. Millar, R.M. Maizels, G. Fabriás, J. Casas, A.H. Buck, Plasmalogen enrichment in exosomes secreted by a nematode parasite versus those derived from its mouse host: implications for exosome stability and biology, Journal of Extracellular Vesicles 5 (2016), https://doi.org/10.3402/jev.v5.30741, 30741.
[66] T. Meningher, G. Lerman, N. Regev-Rudzki, D. Gold, I.Z. Ben-Dov, Y. Sidi, D. Avni, E. Schwartz, Schistosomal MicroRNAs Isolated From Extracellular Vesicles in Sera of Infected Patients: A New Tool for Diagnosis and Follow-up of Human Schistosomiasis, J. Infect. Dis. 215 (2017) 378–386, https://doi.org/10.1093/infdis/jiw539.
[67] V. Samoil, M. Dagenais, V. Ganapathy, J. Aldridge, A. Glebov, A. Jardim, P. Ribeiro, Vesicle-based secretion in schistosomes: Analysis of protein and microRNA (miRNA) content of exosome-like vesicles derived from Schistosoma mansoni, Sci. Rep. 8 (2018), https://doi.org/10.1038/s41598-018-21587-4, 3286.
[68] J. Liu, L. Zhu, J. Wang, L. Qiu, Y. Chen, R.E. Davis, G. Cheng, Schistosoma japonicum extracellular vesicle miRNA cargo regulates host macrophage functions facilitating parasitism, PLoS Pathog. 15 (2019), https://doi.org/10.1371/journal.ppat.1007817 e1007817.
[69] P. Serra, P. Santamaria, Nanoparticle-based approaches to immune tolerance for the treatment of autoimmune diseases, Eur. J. Immunol. 48 (2018) 751–756, https://doi.org/10.1002/eji.201747059.
[70] A.J. Kwiatkowski, J.M. Stewart, J.J. Cho, D. Avram, B.G. Keselowsky, Nano and Microparticle Emerging Strategies for Treatment of Autoimmune Diseases: Multiple Sclerosis and Type 1 Diabetes, Advanced Healthcare Materials 9 (2020), https://doi.org/10.1002/adhm.202000164, 2000164.
[71] E. Montes-Cobos, S. Ring, H.J. Fischer, J. Heck, J. Strauß, M. Schwaninger, S. D. Reichardt, C. Feldmann, F. Lühder, H.M. Reichardt, Targeted delivery of glucocorticoids to macrophages in a mouse model of multiple sclerosis using inorganic-organic hybrid nanoparticles, J Control Release 245 (2017) 157–169, https://doi.org/10.1016/j.jconrel.2016.12.003.
[72] H. Lemos, L. Huang, P.R. Chandler, E. Mohamed, G.R. Souza, L. Li, G. Pacholczyk, G.N. Barber, Y. Hayakawa, D.H. Munn, A.L. Mellor, Activation of the STING Adaptor Attenuates Experimental Autoimmune Encephalitis, J.I 192 (2014) 5571–5578, https://doi.org/10.4049/jimmunol.1303258.
[73] J.M. Gammon, L.H. Tostanoski, A.R. Adapa, Y.-C. Chiu, C.M. Jewell, Controlled delivery of a metabolic modulator promotes regulatory T cells and restrains autoimmunity, J. Control. Release 210 (2015) 169–178, https://doi.org/10.1016/j.jconrel.2015.05.277.
[74] R. Nosratabadi, M. Rastin, M. Sankian, D. Haghmorad, M. Mahmoudi, Hyperforin-loaded gold nanoparticle alleviates experimental autoimmune encephalomyelitis by suppressing Th1 and Th17 cells and upregulating regulatory T cells, Nanomedicine 12 (2016) 1961–1971, https://doi.org/10.1016/j.nano.2016.04.001.
[75] A. Basarkar, J. Singh, Poly (lactide-co-glycolide)-Polymethacrylate Nanoparticles for Intramuscular Delivery of Plasmid Encoding Interleukin-10 to Prevent Autoimmune Diabetes in Mice, Pharm Res 26 (2009) 72–81, https://doi.org/10.1007/s11095-008-9710-4.
[76] B. Phillips, K. Nylander, J. Harnaha, J. Machen, R. Lakomy, A. Styche, K. Gillis, L. Brown, D. Lafreniere, M. Gallo, J. Knox, K. Hogeland, M. Trucco, N. Giannoukakis, A Microsphere-Based Vaccine Prevents and Reverses New-Onset Autoimmune Diabetes, Diabetes 57 (2008) 1544–1555, https://doi.org/10.2337/db07-0507.
[77] D.R. Getts, A.J. Martin, D.P. McCarthy, R.L. Terry, Z.N. Hunter, W.T. Yap, M. T. Getts, M. Pleiss, X. Luo, N.J.C. King, L.D. Shea, S.D. Miller, Microparticles bearing encephalitogenic peptides induce T-cell tolerance and ameliorate experimental autoimmune encephalomyelitis, Nat Biotechnol 30 (2012) 1217–1224, https://doi.org/10.1038/nbt.2434.
[78] R.P. Allen, A. Bolandparvaz, J.A. Ma, V.A. Manickam, J.S. Lewis, Latent, Immunosuppressive Nature of Poly(lactic-co-glycolic acid) Microparticles, ACS Biomater. Sci. Eng. 4 (2018) 900–918, https://doi.org/10.1021/acsbiomaterials.7b00831.
[79] S. Tomić, J. Đokić, S. Vasilijić, N. Ogrinc, R. Rudolf, P. Pelicon, D. Vučević, P. Milosavljević, S. Janković, I. Anžel, Size-dependent effects of gold nanoparticles uptake on maturation and antitumor functions of human dendritic cells in vitro, PLoS One 9 (2014) e96584.
[80] S. Tomić, V. Kokol, D. Mihajlović, A. Mirčić, M. Čolić, Native cellulose nanofibrills induce immune tolerance in vitro by acting on dendritic cells, Sci. Rep. 6 (2016) 1–14.
[81] M. Čolić, S. Tomić, M. Bekić, Immunological aspects of nanocellulose, Immunol. Lett. 222 (2020) 80–89, https://doi.org/10.1016/j.imlet.2020.04.004.
[82] S. Tomić, N. Ilić, V. Kokol, A. Gruden-Movsesijan, D. Mihajlović, M. Bekić, L. Sofronić-Milosavljević, M. Čolić, D. Vučević, Functionalization-dependent effects of cellulose nanofibrils on tolerogenic mechanisms of human dendritic cells, Int J Nanomedicine 13 (2018) 6941–6960, https://doi.org/10.2147/IJN.S183510.
[83] S. Tomić, K. Janjetović, D. Mihajlović, M. Milenković, T. Kravić-Stevović, Z. Marković, B. Todorović-Marković, Z. Spitalsky, M. Micusik, D. Vučević, M. Čolić, V. Trajković, Graphene quantum dots suppress proinflammatory T cell responses via autophagy-dependent induction of tolerogenic dendritic cells, Biomaterials 146 (2017) 13–28, https://doi.org/10.1016/j.biomaterials.2017.08.040.
[84] Rebeka Rudolf, Peter Majerič, Mohammed Shariq, Urban Ferčec, Bojan Budič, Bernd Friedrich, Dragana Vučević, Miodrag Čolić, Morphology, Aggregation Properties, Cytocompatibility, and Anti-Inflammatory Potential of Citrate-Stabilized AuNPs Prepared by Modular Ultrasonic Spray Pyrolysis, Journal of Nanomaterials 2017 (2017), https://doi.org/10.1155/2017/9365012, 9365012.
[85] X. Feng, J. Liu, W. Xu, G. Li, J. Ding, Tackling autoimmunity with nanomedicines, Nanomedicine 15 (2020) 1585–1597, https://doi.org/10.2217/nnm-2020-0102.
[86] F. Cardano, M. Frasconi, S. Giordani, Photo-Responsive Graphene and Carbon Nanotubes to Control and Tackle Biological Systems, Front. Chem. 6 (2018), https://doi.org/10.3389/fchem.2018.00102.
[87] M. Mathiyazhakan, C. Wiraja, C. Xu, A Concise Review of Gold Nanoparticles-Based Photo-Responsive Liposomes for Controlled Drug Delivery, Nano-Micro Lett 10 (2017) 10, https://doi.org/10.1007/s40820-017-0166-0.
[88] I. Ifergan, S.D. Miller, Potential for Targeting Myeloid Cells in Controlling CNS Inflammation, Front. Immunol. 11 (2020), https://doi.org/10.3389/fimmu.2020.571897.
[89] L.K. Prasad, H. O'Mary, Z. Cui, Nanomedicine delivers promising treatments for rheumatoid arthritis, Nanomedicine 10 (2015) 2063–2074, https://doi.org/10.2217/nnm.15.45.
[90] G.D. Vuković, S.Z. Tomić, A.D. Marinković, V. Radmilović, P.S. Uskoković, M. Čolić, The response of peritoneal macrophages to dapsone covalently attached on the surface of carbon nanotubes, Carbon 48 (2010) 3066–3078, https://doi.org/10.1016/j.carbon.2010.04.043.
[91] M. Čolić, T. Džopalić, S. Tomić, J. Rajković, R. Rudolf, G. Vuković, A. Marinković, P. Uskoković, Immunomodulatory effects of carbon nanotubes functionalized with a Toll-like receptor 7 agonist on human dendritic cells, Carbon 67 (2014) 273–287, https://doi.org/10.1016/j.carbon.2013.09.090.
[92] Z. Hunter, D.P. McCarthy, W.T. Yap, C.T. Harp, D.R. Getts, L.D. Shea, S.D. Miller, A Biodegradable Nanoparticle Platform for the Induction of Antigen-Specific Immune Tolerance for Treatment of Autoimmune Disease, ACS Nano 8 (2014) 2148–2160, https://doi.org/10.1021/nn405033r.
[93] A. Gruden-Movsesijan, J. Drulovic, T. Pekmezovic, I. Mitic, J. Cvetkovic, M. Gnjatovic, L. Sofronic-Milosavljevic, Antibodies in sera from multiple sclerosis patients recognize Trichinella spiralis muscle larvae excretory–secretory antigens, Immunobiology 225 (2020), 151954, https://doi.org/10.1016/j.imbio.2020.151954.
[94] X. Clemente-Casares, J. Blanco, P. Ambalavanan, J. Yamanouchi, S. Singha, C. Fandos, S. Tsai, J. Wang, N. Garabatos, C. Izquierdo, S. Agrawal, M.B. Keough, V.W. Yong, E. James, A. Moore, Y. Yang, T. Stratmann, P. Serra, P. Santamaria, Expanding antigen-specific regulatory networks to treat autoimmunity, Nature 530 (2016) 434–440, https://doi.org/10.1038/nature16962.
[95] Y. Chen, J. Wu, J. Wang, W. Zhang, B. Xu, X. Xu, L. Zong, Targeted delivery of antigen to intestinal dendritic cells induces oral tolerance and prevents autoimmune diabetes in NOD mice, Diabetologia 61 (2018) 1384–1396, https://doi.org/10.1007/s00125-018-4593-3.
[96] C.B. Smarr, W.T. Yap, T.P. Neef, R.M. Pearson, Z.N. Hunter, I. Ifergan, D.R. Getts, P.J. Bryce, L.D. Shea, S.D. Miller, Biodegradable antigen-associated PLG nanoparticles tolerize Th2-mediated allergic airway inflammation pre- and postsensitization, Proc. Natl Acad. Sci. 113 (2016) 5059–5064, https://doi.org/10.1073/pnas.1505782113.
[97] R. Kuo, E. Saito, S.D. Miller, L.D. Shea, Peptide-Conjugated Nanoparticles Reduce Positive Co-stimulatory Expression and T Cell Activity to Induce Tolerance, Mol Ther 25 (2017) 1676–1685, https://doi.org/10.1016/j.ymthe.2017.03.032.
[98] N. Filipović, L. Veselinović, S. Ražić, S. Jeremić, M. Filipič, B. Žegura, S. Tomić, M. Čolić, M. Stevanović, Poly (ε-caprolactone) microspheres for prolonged release of selenium nanoparticles, Materials Science and Engineering: C 96 (2019) 776–789, https://doi.org/10.1016/j.msec.2018.11.073.
[99] S. Prasad, T. Neef, D. Xu, J.R. Podojil, D.R. Getts, L.D. Shea, S.D. Miller, Tolerogenic Ag-PLG nanoparticles induce tregs to suppress activated diabetogenic CD4 and CD8 T cells, J Autoimmun 89 (2018) 112–124, https://doi.org/10.1016/j.jaut.2017.12.010.
[100] M. Milosevic, D.B. Stojanovic, V. Simic, M. Grkovic, M. Bjelovic, P.S. Uskokovic, M. Kojic, Preparation and modeling of three-layered PCL/PLGA/PCL fibrous scaffolds for prolonged drug release, Sci. Rep. 10 (2020) 11126, https://doi.org/10.1038/s41598-020-68117-9.
[101] A. Yeste, M. Nadeau, E.J. Burns, H.L. Weiner, F.J. Quintana, Nanoparticle-mediated codelivery of myelin antigen and a tolerogenic small molecule

suppresses experimental autoimmune encephalomyelitis, Proc. Natl Acad. Sci. 109 (2012) 11270–11275, https://doi.org/10.1073/pnas.1120611109.
[102] J.D. Mezrich, J.H. Fechner, X. Zhang, B.P. Johnson, W.J. Burlingham, C. A. Bradfield, An Interaction between Kynurenine and the Aryl Hydrocarbon Receptor Can Generate Regulatory T Cells, J.I 185 (2010) 3190–3198, https://doi.org/10.4049/jimmunol.0903670.
[103] R.A. Maldonado, R.A. LaMothe, J.D. Ferrari, A.-H. Zhang, R.J. Rossi, P.N. Kolte, A.P. Griset, C. O'Neil, D.H. Altreuter, E. Browning, L. Johnston, O.C. Farokhzad, R. Langer, D.W. Scott, U.H. von Andrian, T.K. Kishimoto, Polymeric synthetic nanoparticles for the induction of antigen-specific immunological tolerance, Proc. Natl Acad. Sci. 112 (2015), https://doi.org/10.1073/pnas.1408686111. E156–E165.
[104] B. Tang, X. Liu, M. Liu, X. Bai, Y. Wang, J. Ding, X. Wang, Effects of TLR agonists on immune responses in Trichinella spiralis infected mice, Parasitol Res 119 (2020) 2505–2510, https://doi.org/10.1007/s00436-020-06747-8.
[105] J.J. Cho, J.M. Stewart, T.T. Drashansky, M.A. Brusko, A.N. Zuniga, K.J. Lorentsen, B.G. Keselowsky, D. Avram, An antigen-specific semi-therapeutic treatment with local delivery of tolerogenic factors through a dual-sized microparticle system blocks experimental autoimmune encephalomyelitis, Biomaterials 143 (2017) 79–92, https://doi.org/10.1016/j.biomaterials.2017.07.029.
[106] P. García-González, R. Morales, L. Hoyos, J. Maggi, J. Campos, B. Pesce, D. Gárate, M. Larrondo, R. González, L. Soto, V. Ramos, P. Tobar, M.C. Molina, K. Pino-Lagos, D. Catalán, J.C. Aguillón, A short protocol using dexamethasone and monophosphoryl lipid A generates tolerogenic dendritic cells that display a potent migratory capacity to lymphoid chemokines, J Transl Med 11 (2013), https://doi.org/10.1186/1479-5876-11-128, 128.
[107] K. Otomo, T. Koga, M. Mizui, N. Yoshida, C. Kriegel, S. Bickerton, T.M. Fahmy, G. C. Tsokos, Cutting Edge: Nanogel-Based Delivery of an Inhibitor of CaMK4 to CD4 + T Cells Suppresses Experimental Autoimmune Encephalomyelitis and Lupus-like Disease in Mice, J. Immunol. 195 (2015) 5533–5537, https://doi.org/10.4049/jimmunol.1501603.
[108] S. Tsai, A. Shameli, J. Yamanouchi, X. Clemente-Casares, J. Wang, P. Serra, Y. Yang, Z. Medarova, A. Moore, P. Santamaria, Reversal of autoimmunity by boosting memory-like autoregulatory T cells, Immunity 32 (2010) 568–580, https://doi.org/10.1016/j.immuni.2010.03.015.
[109] S. Singha, K. Shao, Y. Yang, X. Clemente-Casares, P. Solé, A. Clemente, J. Blanco, Q. Dai, F. Song, S.W. Liu, J. Yamanouchi, C.S. Umeshappa, R.H. Nanjundappa, P. Detampel, M. Amrein, C. Fandos, R. Tanguay, S. Newbigging, P. Serra, A. Khadra, W.C.W. Chan, P. Santamaria, Peptide–MHC-based nanomedicines for autoimmunity function as T-cell receptor microclustering devices, Nat. Nanotechnol. 12 (2017) 701–710, https://doi.org/10.1038/nnano.2017.56.
[110] M. Logozzi, D. Mizzoni, B. Bocca, R.D. Raimo, F. Petrucci, S. Caimi, A. Alimonti, M. Falchi, F. Cappello, C. Campanella, C.C. Bavisotto, S. David, F. Bucchieri, D.F. Angelini, L. Battistini, S. Fais, Human primary macrophages scavenge AuNPs and eliminate it through exosomes. A natural shuttling for nanomaterials, European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft für Pharmazeutische Verfahrenstechnik e. V. 137 (2019) 23–36. https://doi.org/10.1016/j.ejpb.2019.02.014.
[111] D.J. Park, W.S. Yun, W.C. Kim, J.-E. Park, S.H. Lee, S. Ha, J.S. Choi, J. Key, Y. J. Seo, Improvement of stem cell-derived exosome release efficiency by surface-modified nanoparticles, Journal of Nanobiotechnology 18 (2020) 178, https://doi.org/10.1186/s12951-020-00739-7.
[112] S. Kumar, I.J. Michael, J. Park, S. Granick, Y.-K. Cho, Cloaked Exosomes: Biocompatible, Durable, and Degradable Encapsulation, Small 14 (2018), 1802052, https://doi.org/10.1002/smll.201802052.
[113] Y. Cao, T. Wu, K. Zhang, X. Meng, W. Dai, D. Wang, H. Dong, X. Zhang, Engineered Exosome-Mediated Near-Infrared-II Region V2C Quantum Dot Delivery for Nucleus-Target Low-Temperature Photothermal Therapy, ACS Nano 13 (2019) 1499–1510, https://doi.org/10.1021/acsnano.8b07224.
[114] M. Khongkow, T. Yata, S. Boonrungsiman, U.R. Ruktanonchai, D. Graham, K. Namdee, Surface modification of gold nanoparticles with neuron-targeted exosome for enhanced blood–brain barrier penetration, Sci. Rep. 9 (2019) 8278, https://doi.org/10.1038/s41598-019-44569-6.