

# The Role of Microparticles as Biomarkers in the Development of Therapy for Autoimmune Disease

David S. Pisetsky

## 1 Introduction

Autoimmune diseases are a diverse group of conditions that result from abnormalities in immune cell function that culminate in tissue inflammation, destruction, or dysfunction [1, 2]. These conditions can cause highly discrete tissue involvement as exemplified by organ-specific autoimmune diseases such as multiple sclerosis or type 1 diabetes or more generalized tissue involvement as exemplified by systemic inflammatory diseases such as systemic lupus erythematosus (SLE). At present, whatever the pattern of tissue involvement, the treatment of the immune component of these diseases utilizes similar agents to either curtail inflammation or to attenuate T and/or B cell reactivity [3]. If damage is irreversible, however, then treatment involves agents that restore to the extent possible the functional impairment that results from unopposed autoimmune attack.

While the etiology and clinical manifestations of autoimmune diseases may vary, the development of new therapies confronts many similar challenges especially in the setting of clinical trials. A particularly serious and vexing challenge relates to biomarkers. For most autoimmune diseases, studies on both patients and animal models have documented a host of phenotypic and functional immune cell abnormalities [4]. Translating these observations into the creation of reliable and actionable markers for use in clinical trials has been difficult, however. Furthermore, as “big data” approaches

---

D.S. Pisetsky, MD, PhD

Division of Rheumatology and Immunology, Department of Medicine, Duke University Medical Center, Durham, NC, USA

Medical Research Service, VA Medical Center, Durham, NC, USA

Duke University Medical Center, Durham VA Medical Center,

508 Fulton Street, 151G, Durham, NC 27705, USA

e-mail: [david.pisetsky@duke.edu](mailto:david.pisetsky@duke.edu)

become more common, the number of biomarkers will undoubtedly rise dramatically; the complexity of these markers will also grow as each marker becomes a composite of thousands or even millions of data points [5–7].

The gap between the biomarkers of today (e.g., C-reactive protein, erythrocyte sedimentation rate, cytokine levels) and those projected for the future (e.g., RNA-seq of individual cells at the site of tissue injury) is enormous, and it will take years before the promise of big data is realized. During the transition from little to big data, the development of new therapies may benefit by some intermediate approaches which can provide a more granular picture of the immune system disturbances associated with autoimmune disease. The value of such approaches will be increased if they can employ instrumentation readily available in most clinical laboratories.

Among these approaches, the analysis of microparticles holds great promise as a platform for the development of new biomarkers to assess the operation of the immune system in autoimmunity. Microparticles (MPs), also called microvesicles (MVs), are small membrane-bound vesicles that are released from activated and dying cells [8]. Following release from cells, MPs enter the blood where their component molecules populate the many “omes” (e.g., proteome, nucleome) that can be identified and quantified by big data. Often these molecules are considered to be free and soluble, whereas, in reality, they are embedded in a particle matrix. In view of the origin of MPs during activation and cell death, particle release is a prominent feature of the pathogenesis of many autoimmune and inflammatory diseases. This chapter will review the structure of particles, their release from cells, and the various ways in which they can be assessed as biomarkers and conceptualized as targets for new therapy.

## 2 Generation of Microparticles

During the normal function of cells as well as the special circumstances of activation and death, cells emit a variety of particle types into the extracellular space. These particles differ in size, composition, and function. As a group, such particles can be termed extracellular vesicles (EVs) [9–11]. The smallest EVs are called exosomes and emanate from the multivesicular bodies on the cell interior. Exosomes are approximately 50–100 nm in diameter. On the other end of the size spectrum, apoptotic bodies are the largest EVs. Apoptotic bodies are the collapsed remnants of dying cells or large fragments that have broken off as death. Given their size and origin, apoptotic bodies can contain a panoply of intracellular constituents albeit in a degraded or rearranged form. Apoptotic bodies can approximate several microns in diameter.

The middle size range of EVs is occupied by MPs. Microparticles range in size from approximately 0.2–1.0  $\mu\text{m}$  and contain a large collection of cellular constituents, including proteins, lipids, and nucleic acids. Importantly, MPs can display bioactive molecules; while the contribution on these molecules to the overall mass may be small, they may contribute importantly to the functional properties of the MPs. MPs result from two seemingly disparate processes: activation and cell death

[8–10]. While this situation may seem paradoxical, in the immune system, activation can lead to cell death, likely as a regulatory strategy to limit or attenuate responses that depend on cellular proliferation.

While the origin of MPs is not fully understood, at least some of these structures may correspond to blebs which arise during apoptosis. Blebs are bubble-like structures that form on or near the cell surface as cells die; blebs may also form during processes such as locomotion as an extension of the cell structure [12, 13]. In the setting of apoptosis, blebs may occur during cell shrinkage to adjust the surface to volume ratio as the cell collapses and dwindles in size. Blebbing may not be simply a physical-chemical response to decreasing volume, however, since there is strong evidence for the role of the ROCK enzyme in their generation [14, 15]. Blebs can occur during early and late apoptosis, and, while the size of blebs and MPs is similar, it is not clear that all MPs come from blebs [16, 17]. Looking at the phenomenon from the perspective of the blebs, it is not clear which blebs leave the cell to enter the extracellular cell space.

A striking feature of blebbing is the translocation of nuclear molecules into these structures as cell death processes proceed [18–22]. Thus, cellular demise during apoptosis involves a regulated process by which nuclear contents are reconfigured and rearranged in a way that fundamentally changes the dying cell's potential interactions with the immune system. The basis of this translocation is speculative although it may facilitate immune clearance of the remnants of dying cells since they are present in a smaller and more “appetizing” form to promote uptake by macrophages. Alternatively, the translocation and associated proteolytic and nucleolytic events may impede processes such as viral or bacterial replication and spread in cases where infection is the proximate cause of the apoptosis.

Whatever the cause of the nuclear translocation, the resulting particles become a rich source of extracellular nuclear molecules. This material includes DNA, RNA, histones, and nonhistone proteins. Indeed, particles are an important source of extracellular nuclear molecules, with their inclusion within the protective space of a membrane-bound structure shielding them from the degradative enzymes present in the blood [23, 24]. Since DNA and RNA are informational macromolecules, their presence in particles points to an important function of particles in the transmission of information from one cell to the next, with microRNA, for example, providing a mechanism for directly modulating cell function. Practically, mining the array of nucleic acids present in MPs as well as exosomes represents a powerful biomarker platform to measure the types of cells that have died and their functional or metabolic state.

### 3 The Assay of Microparticles

Particles are small, with their detection and enumeration presenting significant challenges when these parameters are analyzed by flow cytometry, the current mainstay for these determinations. Flow cytometry performs well for cells but encounters technical difficulties when applied to the submicron size of most particles. Depending

on the instrumentation, particles below 200–500 nm are variably detected. Even this assessment is subject to uncertainty since the size range for detection has been determined on the basis of rigid beads, whereas particles are flexible and indeterminate in shape. Using light scattering for particle detection and logarithmic gain for amplification, flow cytometry can detect many but unlikely not all MPs in a fluid sample. Furthermore, differences in the composition and size array of particles in different biofluids may limit direct comparisons of the properties of particles. For MP analysis, plasma rather than sera is used since blood clotting can lead to particle generation [25–28].

In addition to light scattering, analysis of the “particulome” can utilize many of the same approaches and reagents that are used to analyze cells, recognizing that the small size of particles means that all signals are small and that staining which would make a cell bright will at best lead to staining which is dim. Since MPs have the membrane components from the cell of origin, they bear the characteristic differentiation markers that distinguish lineages. Thus, it is possible to enumerate from the same plasma sample the number of particles from different cell types such as platelets, lymphocytes, or endothelium, all of which are highly relevant to autoimmune disease.

Although measuring events in a particular size range can suffice for counting MPs, some approaches incorporate a further element of staining to assure that MPs are in fact being detected. MPs can arise from apoptotic cells; as such, their membranes have “flipped” and exposed phosphatidylserine (PS) on the surface. Exposed PS is a hallmark of apoptosis and allows identification of apoptotic cells by staining with a fluoresceinated annexin V reagent. While many particles stain positively for annexin V, such positivity is not an invariable feature of particles likely because of the different origins of particles; it is also possible that particles, even from the same cellular source, are heterogeneous in composition and molecular structure [29–31].

Nucleic acids and associated nuclear molecules are important constituents of particles and can be measured by two main methods. Dyes that bind DNA and RNA, such as propidium iodide and SYTO13, can stain particles for enumeration by flow cytometry; the limited amount of material in a small particle and consequent weak signal intensity can challenge this method of detection [32]. In addition, the presence of DNA and other nuclear molecules can be assessed by antibody binding, either a monospecific serum from an autoimmune patient or a monoclonal antibody preparation with a well-defined autoantigen binding [22]. As these nuclear molecules are the targets of antinuclear antibodies (ANAs) in diseases such as lupus, particle assessment is an important element in determining the type and amount of autoreactive material in the blood, recognizing that, in some instances, particles are only one component of this material.

While advances in instrumentation will undoubtedly improve and refine this analysis, at present, flow cytometry can provide information on several aspects of the blood “particulome” that makes this assessment valuable in developing new therapy for autoimmunity, including particle elimination. Table 1 lists these assays and the features which, in some instances, can indicate functional activity of MPs and their putative role in autoimmunity. In comparison to information provided by

**Table 1** Determination of microparticles by functional and cytometric assays

Light scattering to assess particle number
Binding of annexin V to measure exposed phosphatidylserine
Characterization of cell surface markers to define cell of origin
Functional assay for tissue factor
Molecular approaches for enumeration of informational nucleic acids

other biomarkers, even a simple enumeration of MPs can point to the nature of the cells involved in a disease and their physiologic or pathologic state (i.e., activated or dying). Furthermore, the presence in blood of MPs from otherwise inaccessible tissues (e.g., blood vessels) can indicate their possible role in pathogenesis.

## 4 The Functions of Microparticles

The functional activities of MPs are highly varied and consistent with the expression of so many different molecules in these structures, including their surface decoration. As signaling elements, MPs display activities associated with the full size range of immune mediators from the small (e.g., cytokines) to the large (e.g., cell-cell interaction). In general, MPs are pro-inflammatory and pro-thrombotic and can impact on multiple cell types, most prominently, lymphoid and myeloid cells of the immune system and endothelial cells of the vascular system [33–37]. In many respects, MPs can mediate essentially all of the activities that have been considered key to the underlying immune disturbances of autoimmune and inflammatory disease. This possibility should not be surprising since MPs have so many components which are immunologically relevant. The presence of tissue factor is important for the ability of particles to promote thrombosis.

Operationally, distinguishing the functional role of MPs in pathogenesis is difficult since there are few ways at present to either block specifically the activity of MPs or block their production; at this time, inhibition of particle release entails agents that are very broad in activity and likely to affect many other processes (e.g., inhibition of apoptosis, inhibition of activation). While delineating the role of MPs is likely to remain challenging, nevertheless, both *in vivo* and *in vitro* studies clearly demonstrate that MPs can provoke inflammation and thrombosis and therefore can contribute to pathogenesis of autoimmune diseases that are characterized by both of these features [37]. Certainly, the presence of so many bioactive molecules on one structure suggests that MPs can amplify responses by multi-receptor interactions; furthermore, the physical attachment of MPs to another cell type may increase the response of component molecules by increasing the local concentration, causing their transfer or producing repetitive stimulation.

In considering how the functional properties of MPs can impact on autoimmunity, the assay of both MPs and their constituent molecules can strongly influence this assessment. As noted, the assay of MPs requires the use of plasma. In contrast, the assay of cytokines can be accomplished with either plasma or serum. These

fluids differ in composition and the representation of particles. Few studies have directly addressed the differences in levels of various analytes in biofluids. As a result, the contribution of the particle component of an overall cytokine response may be missed. In this regard, certain cytokines (i.e., IL-1 $\beta$ ) can be a component on MPs, with assay of sera possibly missing this important component of the overall response [38]. As the activity of cytokines may be enhanced by its representation on MPs, information of cytokine localization can provide a more complete picture of the potential contribution of a cytokine to disease in comparison to assay of just a biofluid. On the other hand, the contribution of MPs may be missed if particles are bound to cells and are therefore not counted when the cells in plasma are removed during centrifugation. Knowledge of the function of particles is just emerging.

The following sections will focus on two aspects of particle biology that are relevant to autoimmunity.

## 5 MPs as a Source of DAMPs and Alarmins

Microparticles have potentially two important roles in autoimmunity related to their content of nuclear molecules. The first is as a source of alarmins. The second is as a source of nuclear molecules which are the targets of antinuclear antibodies (ANAs) in the context of SLE and related autoimmune disease. As discussed previously, during apoptosis, nuclear molecules undergo translocation as the death process proceeds, with many ultimately residing in blebs. As blebs develop into particles that detach from cells, nuclear molecules can enter into the extracellular space where the particle structure provides a protected environment that may be at least partially resistant to degradation. As some nuclear molecules have immunological activity, MPs have the potential to be important players in pathogenesis.

As shown in many *in vivo* and *in vitro* studies, nuclear molecules can act as damage-associated molecular patterns or DAMPs when they leave their usual intracellular location. A DAMP is an intracellular molecule that can be released from injured, dying, or dead cells and, when in the extracellular space, can exert immunological activity [39, 40]. DAMPs can be large or small molecules and can stimulate immunity by utilizing the same receptors such as the toll-like receptors (TLRs) that are activated by bacterial or viral molecules. These molecules are termed pathogen-associated molecular patterns or PAMPs, while their cognate receptors are called pattern recognition receptors (PRRs). The term alarmin can be applied to certain DAMPs because they alarm the immune system, inducing chemotactic and adjuvant activity. Another term for this group of molecules is danger molecule since they can signal “danger” which represents threats to the organism, including infection or injury, that can induce cell injury.

Among the alarmins, HMGB1 or high-mobility group box 1 protein has potent immune activity that suggests a key role in autoimmunity [41, 42]. HMGB1 is a nonhistone nuclear protein that can bind DNA, mediating processes as a transcription and chromosomal structure. HMGB1 is 215 amino acids long and is comprised

of two DNA binding boxes (A box and B box) as well as a C-terminal tail. This protein is widely expressed in all cells and, while predominantly nuclear in location, can also have cytoplasmic expression. Since all cells can suffer injury and die, HMGB1 has the potential to serve as a uniform danger signal.

HMGB1 can leave cells during activation as well as cell death, with the functional activity resulting from posttranslational modifications (PTMs) that occur during these processes. During activation by TLR agonists such as LPS (endotoxin), or by cytokines, HMGB1 undergoes acetylation; this PTM allows translocation to the cytoplasm for eventual secretion. The presence of acetylation marks the origin of extracellular HMGB1 as activation. Once released from the cell, HMGB1 can serve as a late mediator of endotoxin shock; levels of HMGB1 are increased in conditions such as sepsis, trauma, and malignancy [41–44].

In addition to the occurrence during cell activation, HMGB1 release can occur during different death processes such as apoptosis, necrosis, and pyroptosis. Each of these forms of cell death is distinct in terms of inducing stimulus, a downstream pathway, and ultimately HMGB1 PTMs. Among these PTMs, the redox state is key because of the influence on three sulfhydryl groups at positions 23, 45, and 104. Fully reduced HMGB1 can bind to the chemokine CXCL12 and stimulate chemotaxis. Partially reduced HMGB1, with a disulfhydryl bond between cysteines 23 and 45, can bind to TLR4 and can activate processes in much the same way as does LPS. Fully oxidized HMGB1, as may occur during apoptosis, is inactive. Depending on its biochemistry, HMGB1 can also stimulate cells during TLR2 and RAGE (receptor for advanced glycation end products) [41–44].

As these considerations suggest, HMGB1 release can occur in many of the same situations as does MP release, with both emanating from cells in the seemingly disparate processes of activation and death. Given similarities in the release of HMGB1 and MPs, studies have investigated the presence of HMGB1 on MPs. These studies have assessed HMGB1 content by both flow cytometry and immunoblotting of MPs purified by differential centrifugation; for these studies, particles have come from both blood and cell cultures. Together, these studies demonstrate clearly that HMGB1 can be an important constituent of MPs [45–47]. This finding suggests that at least some of the activities of MPs may result from the presence of a potent alarmin like HMGB1. Because of the importance of PTMs, the presence of HMGB1 on particles does not in and of itself mean that either the HMGB1 is active or that the activity of MPs results from the presence of HMGB1. Determination of the PTMs is necessary to define the activity profile of particle HMGB1.

While HMGB1 can be a component of particles, it can also appear in the blood in a more free or soluble form. Soluble is a relative term that can be defined operationally in terms of behavior during differential sedimentation or size by gel exclusion chromatography. The structure of the more “soluble” form of HMGB1 is not as yet known. Nevertheless, it is possible to assay separately soluble and particle HMGB1 using enzyme-linked immunosorbent assays (ELISA). In this case, the particle and soluble forms can be separated by centrifugation, assaying three sources of HMGB1: uncentrifuged plasma, particle-free or soluble plasma, and sedimented



particles reconstituted to the starting sample volume. Flow cytometry can complement the immunochemical assay of these preparations.

Analysis of HMGB1 by these approaches shows a number of important features relevant to the role of HMGB1 as a disease mediator as well as a biomarker in autoimmunity. First, HMGB1 in the blood can exist in a free- and particle-bound component. Second, changes in the expression of HMGB1 may be detected by assay of HMGB1 on particles by flow cytometry that may not be apparent in the ELISA. In a study of normal volunteers receiving a low dose of LPS systemically, changes in the number of particles positive for HMGB1 were observed by flow cytometry, whereas the levels by ELISA were unchanged [48]. These findings suggest that analysis of MP levels of alarmins may provide more sensitive detection of HMGB1 in time-course studies than the overall protein levels in unfractionated or uncentrifuged plasma.

Another finding that emerges from analysis of MP levels relates to quantitation of the overall magnitude of the response. As shown in preliminary experiments, the amount of HMGB1 measured in the combination of the soluble and particle components can be greater than the amount measured in the uncentrifuged plasma (unpublished observations). While the explanation for this finding is not known, it is possible that the physical process of separation can reveal or unmask HMGB1 that is ordinarily present on the interior of the particle and thus unavailable for detection in an immunoassay. Sample handling, especially the mechanical forces that occur with high-speed centrifugation, may disrupt particle structure or cause fragmentation to increase the availability of this interior component.

Many molecules in the blood are putatively “soluble” and assayed as biomarkers for damage or death of cells (e.g., ALT and AST for the liver and troponin for the heart) or activation of cells (e.g., soluble IL-2 receptor). Serum is the usual source of blood for assay and the existence of a particle component is not generally considered. With the precedent of particle-bound HMGB1 in mind, subsequent studies explored the representation of soluble CD40 ligand (sCD40L) in a particle and free form. sCD40L is a transmembrane protein found on T cells and platelets, with its soluble form assayed as a biomarker for inflammatory and thrombotic disorders. Using blood from the same population of volunteers given LPS, studies demonstrated that sCD40L also exists on particles, with assay of the particle-bound form providing information not apparent with assay of the soluble form [49]. Thus, these studies indicate that solubility in the context of the biomarker studies on blood does not signify an intrinsic physical-chemical property but rather an operational property dependent on the handling of specimens.

## 6 Microparticles as a Source of Immune Complexes

The formation of immune complexes (ICs) is a central event in the pathogenesis of many autoimmune and inflammatory diseases. These ICs can occur in blood as in the case of SLE or can occur in local spaces such as the joint in rheumatoid arthritis



(RA). ICs have several distinct roles in the pathogenesis: they can deposit in the tissue to activate complement and promote tissue injury (i.e., lupus nephritis); they can activate complement to induce local inflammation (i.e., rheumatoid synovium); and, depending on the target antigens in the ICs, they can induce cytokine production by cells of the innate immune system, most prominently, the plasmacytoid dendritic cells (PDCs) [50–54]. The latter mechanism is particularly important in SLE since the ICs contain nuclear molecules. While nuclear molecules like HMGB1 appear active when alone, DNA does not induce immune response unless bound by a protein such as an autoantibody.

The enhanced activity of DNA in immune complexes likely results from the uptake of complexes into the cells in a manner that exposes the DNA to internal nucleic acid sensors [55]. These sensors include molecules such as TLR9 and cGAS and represent an internal defense system which likely has evolved to meet the challenge of intracellular infection, whether bacterial or viral. Incubation of cells such as macrophages with DNA does not allow access to the subcellular compartment in the cytoplasm where these receptors are located. In contrast, an IC can essentially transfect DNA into the cytoplasm where it can mimic intracellular DNA from an infection and trigger cell activation. This mechanism can lead to the production of cytokines such as type 1 interferon whose molecular signature is a hallmark of lupus pathogenesis.

The evidence for the role of ICs in lupus pathogenesis is strong, with depression of complement levels and increases in complement split products demonstrating the existence of complexes somewhere in the body. While blood is the obvious place to look for ICs, they have been in fact difficult to demonstrate by biochemical and immunochemical techniques in lupus. Two main explanations have been invoked to explain this difficulty: the formation of ICs *in situ* in tissue (e.g., kidney) rather than blood and rapid clearance or deposition of ICs such that their presence in blood is ephemeral or otherwise undetectable [54]. Given the centrality of ICs to the pathogenesis of lupus nephritis and interferon production, the absence of a direct measure of their presence has deprived the field of a critical biomarker.

As in the case of molecules that are putatively soluble, complexes, which are also generally considered soluble, may in fact be particulate. Furthermore, in view of evidence that MPs can contain the nuclear antigens targeted by ANAs, a role of particles as a source of ICs becomes very plausible. Studies have therefore investigated MPs as a source of ICs critical to lupus, and several lines of evidence are very consistent with this possibility. Thus, ANAs, either sera from patients or lupus mice as well as murine monoclonal antibodies, can all bind to particles that have been generated *in vitro* from cell lines treated with agents that cause activation or cell death [22, 56–58]. The binding is not invariable, however, reflecting either the fine specificity of the antibodies as well as the amount and the extent of surface expression of the target antigens.

Most importantly, particles from the blood of patients with lupus and certain strains of autoimmune mice contain bound IgG at levels that far exceed those of control particles from healthy individuals or mice. The presence of such IgG can be demonstrated by flow cytometric techniques as well as proteomic analysis. Since levels of

**Table 2** Role of MPs in the pathogenesis of autoimmunity

Stimulate inflammation via constituent cytokines
Stimulate inflammation via constituent alarmins
Promote thrombosis
Form immune complexes with tissue deposition
Transfer information via constituent nucleic acids

IgG on particles can be related to levels of anti-DNA, these findings suggest that particle DNA can be an important source of antigen for the formation of ICs [59–61]. Indeed, the finding of IgG on MPs provides some of the most decisive evidence for the presence of circulating ICs in the blood of lupus patients and points to the utility of this assessment for biomarker purposes. The demonstration of a microparticle component in renal biopsies of patients with lupus nephritis supports this view [61].

Similar studies point to a role of MPs in IC formation in rheumatoid arthritis although these studies suggest that local factors may determine this process [62, 63]. Interestingly, particles in synovial fluid of patients with RA bear IgG as well as complement components demonstrating directly that MPs can be IC components. In contrast, the plasmas of the same patients did not show increased numbers of IgG positive particles. These observations could suggest differences in the antigenic content of MPs that either form in the joint space or localize there. Alternatively, autoantibody synthesis in the synovium may lead to sufficient amounts to coat particles, whereas dilution of these antibodies in the blood may prevent appreciable particle binding. Antibodies to citrullinated proteins (ACPAs) are the likely specificity contributing to the formation of these complexes, perhaps by binding proteins that have undergone citrullination.

Elucidation of the role of MP complexes in other autoimmune diseases is just beginning although, given the content of self-molecules in MPs, these structures could represent a common nidus for IC formation. Particles, whether coated with autoantibodies and complement, could also act in many other ways in the disease setting. Table 2 summarizes potential roles of MPs in the pathogenesis of autoimmunity.

## 7 Implications for New Therapies

MPs are newly recognized players in the pathogenesis of autoimmunity and therefore can serve as both biomarkers and targets of therapy. The biomarker potential of MP assessment is high since their analysis in blood can provide a window to observe events in the periphery including activation and death of cells in locations such as the vasculature. Since assays involve relatively small amounts of blood, analysis of changes over time to monitor disease activity or the response to treatment can be readily accomplished. Such assessment can be quantitative although current technology may not provide a full and completely accurate picture of the number and array of particles present. Nevertheless, the detail captured in this picture can exceed that currently available from other approaches [64].

The analysis of MP ICs found in plasma represents an entirely new approach for characterizing the vasculature in autoimmune disease. Studies in the context of atherosclerosis, diabetes, and metabolic syndrome have clearly demonstrated the value of particle assessment in developing predictive markers for events such as cardiac ischemia and stroke [65–70]. Importantly, the presence of endothelial MPs in blood allows analysis of the state of the endothelium in these conditions, characterizing particles using different cell surface markers associated with their physiologic state [71]. Since atherosclerosis involves localized inflammation of the plaque in the vessel wall, analysis of immune cell properties of circulating MPs can augment any information provided by nonspecific markers such as C-reactive protein.

Since many autoimmune diseases have an increased frequency of atherosclerosis, analysis of MPs can provide a simultaneous assessment of the immune system and vascular system. In this regard, particles have pro-thrombotic properties, with analysis of their number and properties potentially providing predictive information on thrombotic events which can involve a variety of organ systems in autoimmune disease. Studies in oncology have explored the value of this type of assessment since thrombosis is an important complication of many malignancies; as in the case of cardiovascular disease, cancer is a setting for high levels of particles in the blood [72].

At present, the link between inflammation and vascular disease is not well understood nor are the effects of current treatments on the risk for cardiac events. As the armamentarium of new immunomodulatory agents grows along with the number of combinations between new and existing agents, it will be important to have markers that could be useful in distinguishing effects on cardiac risk compared to other inflammatory disease manifestations such as synovitis or glomerulonephritis. The sensitivity of MP assessment in comparison to noninvasive tests of cardiovascular disease (e.g., flow-mediated dilatation) is an exciting area of future research that could provide unique biomarker information to sort out the effects of treatment on different target tissues [73].

The assessment of MPs occurs at the junction of little and big data. The little data aspect involves a simple count of particle types. The big data aspect involves a detailed analysis of the constituent molecules of the particles—proteins, lipids, nucleic acids—by array or omics techniques. In particular, MPs can provide a unique source of RNA for analysis of both messenger and microRNA species. Such an analysis would clearly place MP studies in the big data arena although the advantage of MP assessment in comparison to that of total blood comes from knowledge of the cell of origin of any RNA in the blood. Since MP can be separated by flow cytometric techniques on the basis of phenotype, an analysis of their macromolecular composition may allow determination of events in even uncommon or rare cell populations, possibly including those critically involved in pathogenesis.

At present, the main limitation in the study of MPs as biomarkers is technical and relates to the small size of particles. Even with the best instruments, the total counting of particles is uncertain given their size [74]. Furthermore, as particles are small, detection of certain cell populations on the basis of their differentiation markers may be insensitive especially if the density of the marker is low or the detecting antibody produces a weak binding or a weak signal. Development of more sensitive

**Table 3** Advantages of MPs as biomarkers

Information on a wide range of cell types from a single sample
Reveal events in rare or inaccessible cell populations (e.g., endothelium)
Delineate pathogenic processes (e.g., activation or cell death)
Provide a source of material from a single cell type for omics assay
Reveal changes of diverse cell types with treatment and disease activity

particle assays will therefore be important in exploiting more fully the potential of MP assessment as a platform for novel biomarkers. Table 3 lists advantages of MP assessment for biomarker purposes.

The targeting of MPs for therapy would represent a fundamentally new direction in the treatment of autoimmune disease although, as noted, agents such as anti-cytokines and anti-HMGB1 may in fact work by interdicting molecules on particles. Similarly, agents designed to block IC formation or promote IC dissolution could be explored whether the IC is soluble or particulate [75]. On the other hand, strategies to prevent particle release by specific blockade of the steps in particle formation could have therapeutic applicability although the development of such approaches requires understanding of not only the actual processes of particle formation and release but also their physiological consequences [76, 77]. Studies in a number of disease settings have demonstrated reduction of MP levels with a variety of treatment; whether these effects are primary or secondary is not known, however.

If particles are simple by-products of other processes, their production could at least be theoretically blocked without interfering with cell function. If, however, particle release is integral to some critical process (e.g., detoxification or removal of damaged subcellular organelles), then its blockade could have adverse effects. In this regard, if particle release is essential to the response to danger, inhibition of this process could impair host defense and increase susceptibility to infection. At present, these considerations are speculative and point to the many unknown aspects of particle biology.

## 8 Conclusions

Microparticles are small membrane-bound vesicles that carry intracellular molecules into the extracellular space and exert many important biological activities. The potential role of these structures in the pathogenesis of autoimmune disease is very high since MPs can promote both inflammation and thrombosis. At present, MPs represent novel biomarkers to measure disease activity and the functional status of diverse cell populations, expanding the perspective currently available for noninvasive assessment of steps essential for pathogenesis. Future studies will determine whether MPs can also be a target of therapy, with their elimination or functional inactivation a promising avenue for next-generation treatments.

## References

1. Davidson A, Diamond B (2001) Autoimmune diseases. *N Engl J Med* 345:340–350
2. Rosenblum MD, Remedios KA, Abbas AK (2015) Mechanisms of human autoimmunity. *J Clin Investig* 125:2228–2233
3. Thanou A, Merrill JT (2014) Treatment of systemic lupus erythematosus: new therapeutic avenues and blind alleys. *Nat Rev Rheumatol* 10:23–34
4. Mohan C, Assassi S (2015) Biomarkers in rheumatic diseases: how can they facilitate diagnosis and assessment of disease activity? *BMJ* 351:h5079
5. Chiche L, Jourde-Chiche N, Whalen E, Presnell S, Gersuk V et al (2014) Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. *Arthritis Rheumatol* 66:1583–1595
6. Dennis G Jr, Holweg CT, Kummerfeld SK, Choy DF, Setiadi AF et al (2014) Synovial phenotypes in rheumatoid arthritis correlate with response to biologic therapeutics. *Arthritis Res Ther* 16:R90
7. Housley WJ, Fernandez SD, Vera K, Murikinati SR, Grutzendler J et al (2015) Genetic variants associated with autoimmunity drive NF $\kappa$ B signaling and responses to inflammatory stimuli. *Sci Transl Med* 7:291ra293
8. Beyer C, Pisetsky DS (2010) The role of microparticles in the pathogenesis of rheumatic diseases. *Nat Rev Rheumatol* 6:21–29
9. Gould SJ, Raposo G (2013) As we wait: coping with an imperfect nomenclature for extracellular vesicles. *J Extracell Vesicles* 2: 20389
10. Buzas EI, Gyorgy B, Nagy G, Falus A, Gay S (2014) Emerging role of extracellular vesicles in inflammatory diseases. *Nat Rev Rheumatol* 10:356–364
11. Minciacchi VR, Freeman MR, Di Vizio D (2015) Extracellular vesicles in cancer: exosomes, microvesicles and the emerging role of large oncosomes. *Semin Cell Dev Biol* 40:41–51
12. Charras GT (2008) A short history of blebbing. *J Microsc* 231:466–478
13. Charras GT, Coughlin M, Mitchison TJ, Mahadevan L (2008) Life and times of a cellular bleb. *Biophys J* 94:1836–1853
14. Coleman ML, Sahai EA, Yeo M, Bosch M, Dewar A et al (2001) Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I. *Nat Cell Biol* 3:339–345
15. Sebbagh M, Renvoize C, Hamelin J, Riche N, Bertoglio J et al (2001) Caspase-3-mediated cleavage of ROCK I induces MLC phosphorylation and apoptotic membrane blebbing. *Nat Cell Biol* 3:346–352
16. Wickman GR, Julian L, Mardilovich K, Schumacher S, Munro J et al (2013) Blebs produced by actin-myosin contraction during apoptosis release damage-associated molecular pattern proteins before secondary necrosis occurs. *Cell Death Differ* 20:1293–1305
17. Zirngibl M, Furnrohr BG, Janko C, Munoz LE, Voll RE et al (2015) Loading of nuclear autoantigens prototypically recognized by systemic lupus erythematosus sera into late apoptotic vesicles requires intact microtubules and myosin light chain kinase activity. *Clin Exp Immunol* 179:39–49
18. Casciola-Rosen LA, Anhalt G, Rosen A (1994) Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J Exp Med* 179:1317–1330
19. Halicka HD, Bedner E, Darzynkiewicz Z (2000) Segregation of RNA and separate packaging of DNA and RNA in apoptotic bodies during apoptosis. *Exp Cell Res* 260:248–256
20. Lane JD, Allan VJ, Woodman PG (2005) Active relocation of chromatin and endoplasmic reticulum into blebs in late apoptotic cells. *J Cell Sci* 118:4059–4071
21. Schiller M, Bekeredjian-Ding I, Heyder P, Blank N, Ho AD et al (2008) Autoantigens are translocated into small apoptotic bodies during early stages of apoptosis. *Cell Death Differ* 15:183–191
22. Reich CF 3rd, Pisetsky DS (2009) The content of DNA and RNA in microparticles released by Jurkat and HL-60 cells undergoing in vitro apoptosis. *Exp Cell Res* 315:760–768

23. Pisetsky DS, Gauley J, Ullal AJ (2011) Microparticles as a source of extracellular DNA. *Immunol Res* 49:227–234
24. Dye JR, Ullal AJ, Pisetsky DS (2013) The role of microparticles in the pathogenesis of rheumatoid arthritis and systemic lupus erythematosus. *Scand J Immunol* 78:140–148
25. Dragovic RA, Gardiner C, Brooks AS, Tannetta DS, Ferguson DJ et al (2011) Sizing and phenotyping of cellular vesicles using nanoparticle tracking analysis. *Nanomedicine* 7:780–788
26. Robert S, Poncelet P, Lacroix R, Raoult D, Dignat-George F (2011) More on: calibration for the measurement of microparticles: value of calibrated polystyrene beads for flow cytometry-based sizing of biological microparticles. *J Thromb Haemost* 9:1676–1678; author reply 1681–1672
27. van der Pol E, Coumans FA, Grootemaat AE, Gardiner C, Sargent IL et al (2014) Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing. *J Thromb Haemost* 12:1182–1192
28. Poncelet P, Robert S, Bailly N, Garnache-Ottou F, Bouriche T et al (2015) Tips and tricks for flow cytometry-based analysis and counting of microparticles. *Transfus Apher Sci* 53:110–126
29. Connor DE, Exner T, Ma DD, Joseph JE (2010) The majority of circulating platelet-derived microparticles fail to bind annexin V, lack phospholipid-dependent procoagulant activity and demonstrate greater expression of glycoprotein Ib. *Thromb Haemost* 103:1044–1052
30. Frey B, Gaipf US (2011) The immune functions of phosphatidylserine in membranes of dying cells and microvesicles. *Semin Immunopathol* 33:497–516
31. Nicolai E, Squatrito D, Emmi G, Silvestri E, Emmi L et al (2015) A new cytofluorimetric approach to evaluate the circulating microparticles in subjects with antiphospholipid antibodies. *Thromb Res* 136:1252–1258
32. Ullal AJ, Pisetsky DS, Reich CF 3rd (2010) Use of SYTO 13, a fluorescent dye binding nucleic acids, for the detection of microparticles in in vitro systems. *Cytom A* 77:294–301
33. Aras O, Shet A, Bach RR, Hysjulien JL, Slungaard A et al (2004) Induction of microparticle- and cell-associated intravascular tissue factor in human endotoxemia. *Blood* 103:4545–4553
34. Morel O, Toti F, Hugel B, Freyssinet JM (2004) Cellular microparticles: a disseminated storage pool of bioactive vascular effectors. *Curr Opin Hematol* 11:156–164
35. Angelillo-Scherrer A (2012) Leukocyte-derived microparticles in vascular homeostasis. *Circ Res* 110:356–369
36. van Es N, Bleker S, Sturk A, Nieuwland R (2015) Clinical significance of tissue factor-exposing microparticles in arterial and venous thrombosis. *Semin Thromb Hemost* 41:718–727
37. Yanez-Mo M, Siljander PR, Andreu Z, Zavec AB, Borrás FE et al (2015) Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles* 4:27066
38. Prada I, Furlan R, Matteoli M, Verderio C (2013) Classical and unconventional pathways of vesicular release in microglia. *Glia* 61:1003–1017
39. Pisetsky D (2011) Cell death in the pathogenesis of immune-mediated diseases: the role of HMGB1 and DAMP-PAMP complexes. *Swiss Med Wkly* 141:w13256
40. Venereau E, Ceriotti C, Bianchi ME (2015) DAMPs from cell death to new life. *Front Immunol* 6:422
41. Harris HE, Andersson U, Pisetsky DS (2012) HMGB1: a multifunctional alarmin driving auto-immune and inflammatory disease. *Nat Rev Rheumatol* 8:195–202
42. Andersson U, Antoine DJ, Tracey KJ (2014) The functions of HMGB1 depend on molecular localization and post-translational modifications. *J Intern Med* 276:420–424
43. Venereau E, Casalgrandi M, Schiraldi M, Antoine DJ, Cattaneo A et al (2012) Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. *J Exp Med* 209:1519–1528
44. Yang H, Antoine DJ, Andersson U, Tracey KJ (2013) The many faces of HMGB1: molecular structure-functional activity in inflammation, apoptosis, and chemotaxis. *J Leukoc Biol* 93:865–873



45. Maugeri N, Rovere-Querini P, Baldini M, Baldissera E, Sabbadini MG et al (2014) Oxidative stress elicits platelet/leukocyte inflammatory interactions via HMGB1: a candidate for microvessel injury in systemic sclerosis. *Antioxid Redox Signal* 20:1060–1074
46. Pisetsky DS (2014) The expression of HMGB1 on microparticles released during cell activation and cell death in vitro and in vivo. *Mol Med* 20:158–163
47. Spencer DM, Mobarrez F, Wallen H, Pisetsky DS (2014) The expression of HMGB1 on microparticles from Jurkat and HL-60 cells undergoing apoptosis in vitro. *Scand J Immunol* 80:101–110
48. Soop A, Hallstrom L, Frostell C, Wallen H, Mobarrez F et al (2013) Effect of lipopolysaccharide administration on the number, phenotype and content of nuclear molecules in blood microparticles of normal human subjects. *Scand J Immunol* 78:205–213
49. Mobarrez F, Sjovik C, Soop A, Hallstrom L, Frostell C, et al. (2015) CD40L expression in plasma of volunteers following LPS administration: A comparison between assay of CD40L on platelet microvesicles and soluble CD40L. *Platelets*: 26:486–490
50. Koffler D, Agnello V, Thoburn R, Kunkel HG (1971) Systemic lupus erythematosus: prototype of immune complex nephritis in man. *J Exp Med* 134:169s–179s
51. Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ et al (2002) Chromatin-IgG complexes activate B cells by dual engagement of IgM and toll-like receptors. *Nature* 416:603–607
52. Lovgren T, Eloranta ML, Bave U, Alm GV, Ronnblom L (2004) Induction of interferon-alpha production in plasmacytoid dendritic cells by immune complexes containing nucleic acid released by necrotic or late apoptotic cells and lupus IgG. *Arthritis Rheum* 50:1861–1872
53. Tian J, Avalos AM, Mao SY, Chen B, Senthil K et al (2007) Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nat Immunol* 8:487–496
54. Sereckina N, Van Der Vlag J, Berden J, Mortensen E, Rekvig OP (2013) Lupus nephritis: enigmas, conflicting models and an emerging concept. *Mol Med* 19:161–169
55. Kawasaki T, Kawai T, Akira S (2011) Recognition of nucleic acids by pattern-recognition receptors and its relevance in autoimmunity. *Immunol Rev* 243:61–73
56. Ullal AJ, Reich CF 3rd, Clowse M, Criscione-Schreiber LG, Tochacek M et al (2011) Microparticles as antigenic targets of antibodies to DNA and nucleosomes in systemic lupus erythematosus. *J Autoimmun* 36:173–180
57. Ullal AJ, Pisetsky DS (2013) The role of microparticles in the generation of immune complexes in murine lupus. *Clin Immunol* 146:1–9
58. Ullal AJ, Marion TN, Pisetsky DS (2014) The role of antigen specificity in the binding of murine monoclonal anti-DNA antibodies to microparticles from apoptotic cells. *Clin Immunol* 154:178–187
59. Nielsen CT, Ostergaard O, Stener L, Iversen LV, Truedsson L et al (2012) Increased IgG on cell-derived plasma microparticles in systemic lupus erythematosus is associated with autoantibodies and complement activation. *Arthritis Rheum* 64:1227–1236
60. Ostergaard O, Nielsen CT, Iversen LV, Tanassi JT, Knudsen S et al (2013) Unique protein signature of circulating microparticles in systemic lupus erythematosus. *Arthritis Rheum* 65:2680–2690
61. Nielsen CT, Ostergaard O, Rekvig OP, Sturfelt G, Jacobsen S et al (2015) Galectin-3 binding protein links circulating microparticles with electron dense glomerular deposits in lupus nephritis. *Lupus* 24:1150–1160
62. Biro E, Nieuwland R, Tak PP, Pronk LM, Schaap MC et al (2007) Activated complement components and complement activator molecules on the surface of cell-derived microparticles in patients with rheumatoid arthritis and healthy individuals. *Ann Rheum Dis* 66:1085–1092
63. Cloutier N, Tan S, Boudreau LH, Cramb C, Subbaiah R et al (2013) The exposure of autoantigens by microparticles underlies the formation of potent inflammatory components: the microparticle-associated immune complexes. *EMBO Mol Med* 5:235–249
64. Franca CN, Izar MC, Amaral JB, Tegani DM, Fonseca FA (2015) Microparticles as potential biomarkers of cardiovascular disease. *Arq Bras Cardiol* 104:169–174



65. Omoto S, Nomura S, Shouzu A, Nishikawa M, Fukuhara S et al (2002) Detection of monocyte-derived microparticles in patients with Type II diabetes mellitus. *Diabetologia* 45:550–555
66. Nomura S, Shouzu A, Omoto S, Nishikawa M, Iwasaka T (2005) Long-term treatment with nifedipine modulates procoagulant marker and C-C chemokine in hypertensive patients with type 2 diabetes mellitus. *Thromb Res* 115:277–285
67. Nozaki T, Sugiyama S, Koga H, Sugamura K, Ohba K et al (2009) Significance of a multiple biomarkers strategy including endothelial dysfunction to improve risk stratification for cardiovascular events in patients at high risk for coronary heart disease. *J Am Coll Cardiol* 54:601–608
68. Lee ST, Chu K, Jung KH, Kim JM, Moon HJ et al (2012) Circulating CD62E+ microparticles and cardiovascular outcomes. *PLoS One* 7:e35713
69. Sarlon-Bartoli G, Bennis Y, Lacroix R, Piercecchi-Marti MD, Bartoli MA et al (2013) Plasmatic level of leukocyte-derived microparticles is associated with unstable plaque in asymptomatic patients with high-grade carotid stenosis. *J Am Coll Cardiol* 62:1436–1441
70. Wekesa AL, Cross KS, O'Donovan O, Dowdall JF, O'Brien O et al (2014) Predicting carotid artery disease and plaque instability from cell-derived microparticles. *Eur J Vasc Endovasc Surg* 48:489–495
71. Dignat-George F, Boulanger CM (2011) The many faces of endothelial microparticles. *Arterioscler Thromb Vasc Biol* 31:27–33
72. Pabinger I, Thaler J, Ay C (2013) Biomarkers for prediction of venous thromboembolism in cancer. *Blood* 122:2011–2018
73. Augustine D, Ayers LV, Lima E, Newton L, Lewandowski AJ et al (2014) Dynamic release and clearance of circulating microparticles during cardiac stress. *Circ Res* 114:109–113
74. Robert S, Lacroix R, Poncelet P, Harhoury K, Bouriche T et al (2012) High-sensitivity flow cytometry provides access to standardized measurement of small-size microparticles – brief report. *Arterioscler Thromb Vasc Biol* 32:1054–1058
75. Stearns NA, Lee J, Leong KW, Sullenger BA, Pisetsky DS (2012) The inhibition of anti-DNA binding to DNA by nucleic acid binding polymers. *PLoS One* 7:e40862
76. Morel O, Jesel L, Freyssinet JM, Toti F (2011) Cellular mechanisms underlying the formation of circulating microparticles. *Arterioscler Thromb Vasc Biol* 31:15–26
77. Roseblade A, Luk F, Rawling T, Ung A, Grau GE et al (2013) Cell-derived microparticles: new targets in the therapeutic management of disease. *J Pharm Pharm Sci* 16:238–253