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Comment on György et al, page e39

# MPs or ICs?

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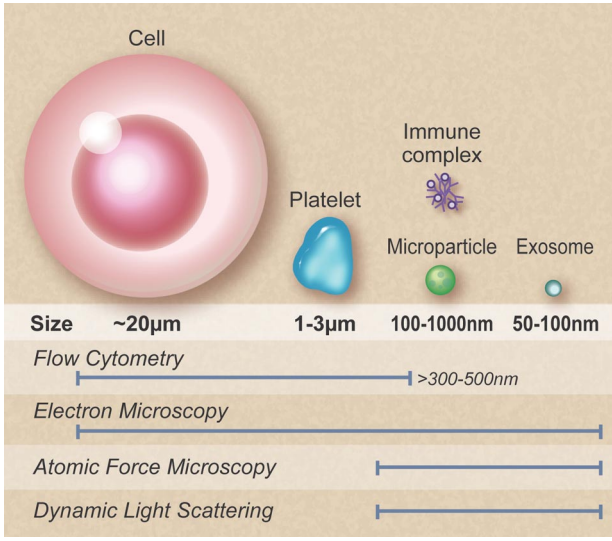
In this issue of *Blood*, György and colleagues used multiple methods to characterize cell-derived microparticles (MPs) in the plasma and synovial fluid of arthritis patients and discovered that MPs and immune complexes (ICs) have overlapping biophysical properties (see figure).[1]




**Cells, MPs, and exosomes. Sizes of the different cells and particles and techniques used to study them are shown. (Professional illustration by Debra T. Dartez.)**

MPs (also called microvesicles) are a heterogeneous population of membrane vesicles ranging between 100 and 1000 nm in diameter that are released from activated and apoptotic cells.[2] They are present in various body fluids, such as plasma, synovial fluid, and urine.[3] MPs have cell-surface receptors, cytosolic proteins, DNA, mRNA, and microRNA derived from their cell of origin and act as vectors of cell-cell communication.[2,3] MP levels are increased in various diseases and have been proposed to play roles in many processes, including inflammation, thrombosis, and angiogenesis.[2,3] They may also be useful biomarkers of disease.

Various methods are used to analyze the biophysical properties of MPs, including flow cytometry, dynamic light scattering, atomic force microscopy, and electron microscopy. In addition, functional properties of MPs, such as their procoagulant activity, can be assessed using different assays. Each method has strengths and weaknesses.

The majority of studies use flow cytometry to characterize MPs in clinical samples. The strengths of this method are that it allows enumeration and determination of the cell origin. It is generally thought that flow cytometers can measure larger MPs (~ 300-1000 nm; see figure). It should be noted that it is critical that MPs be labeled with directly conjugated antibodies and/or annexin V (which binds to phosphatidylserine exposed on the surface of many MPs). Early studies reported a 40-fold range in the number of platelet MPs in the plasma of healthy subjects. In response to this high level of variability, efforts have begun to standardize the quantification of MPs by flow cytometry. The first advance was the introduction of fluorescent-calibrated submicrometer beads. Indeed, newer flow cytometers can now distinguish 100- and 300-nm beads.[4,5] However, these calibration beads have fundamentally different properties of forward and side scatter compared with biologic MPs, and the lower limit of detection of biologic MPs by flow cytometry is currently unclear. Despite these advances in analysis of MPs by flow cytometry, inherent limitations of this approach remain because most machines only analyze the larger MPs.

Dynamic light scattering, atomic force microscopy, and electron microscopy can be used to measure small particles, such as MPs and exosomes (see figure). Dynamic light scattering is excellent for determining size and quantification of particles but provides no information on the properties of the particle.[4] In addition, it is more suited for analyzing homogeneous populations of particles rather than heterogeneous MP populations. Atomic force microscopy and electron microscopy allow for accurate determination of particle size and shape, but cannot be used for routine analysis of clinical samples.[5] Lacroix and colleagues have recently described the strengths and weaknesses of the different techniques to measure MPs.[6]

MPs are increased in the synovial fluid of rheumatoid arthritis patients and likely play a role in inflammation.[7] A strength of the study by György and colleagues is that it used a combination of methods to analyze MPs.[1] They found that MPs and ICs had similar biophysical properties, including size and light scattering. For instance, analysis by dynamic light scattering and atomic force microscopy

showed that insoluble ICs and MPs both have sizes of 100-200 nm in diameter. Using flow cytometry, ICs were present in the forward scatter by side scatter gate used to define MPs. MPs and ICs could not be separated by differential centrifugation. However, the authors demonstrated that MPs from synovial fluid and plasma could be solubilized with detergent that did not affect ICs. One limitation of the study is that it used an older-style FACSCalibur flow cytometer.[1] Nonetheless, these results suggest that previous studies, which did not discriminate MPs and ICs in the synovial fluid of rheumatoid arthritis patients, might have mistaken ICs for MPs.

The findings by György and colleagues highlight the limitations in the current techniques used to study MPs (when used alone) and raise the possibility that what is termed a MP in various body fluids may represent some other biologic or nonbiologic particles.[1] Despite the excitement about MPs and their multiple roles in different biologic processes, there has been little attention paid to the techniques that are used to characterize and quantify these small particles. Variability in the measurement of MPs may also be due to a lack of standardization of preanalytical variables, such as collection of samples, storage, and centrifugation speeds.[8,9] The development of new techniques and instruments, such as impedance-based flow cytometry and Nanosight tracking analysis, and the standardization of preanalytical and analytical variables to measure MPs, should greatly advance the field.

*Conflict-of-interest disclosure: The authors declare no competing financial interests.* ■

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Comment on Roger et al, page 1205

# HDAC inhibitors block innate immunity

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In this issue of *Blood*, Roger and colleagues present data on the magnitude of influence that broad-spectrum HDAC inhibitors exert on TLR-driven immune responses, thus demonstrating that HDAC inhibitors are immunosuppressive drugs.

Histone deacetylase (HDAC) inhibitors have become promising candidates for the treatment of different types of cancer. "At least 80 clinical trials are under way, testing more than eleven different HDAC inhibitory agents,"[1p1] for their antitumor effect in hematologic and solid malignancies. The HDAC inhibitor vorinostat is now an approved add-on therapy for cutaneous T-cell lymphoma.[2] HDAC inhibitors induce growth arrest, differentiation, and programmed cell death, and inhibit invasion and angiogenesis. However, over the years, evidence has accumulated showing that HDAC inhibitors also have immunomodulatory activity even in non-apoptotic concentrations. Although HDAC inhibitors increase acetylation of histones, a condition associated with increased transcriptional accessibility, multiple reports have shown that HDAC inhibitors possess suppressive effects on immune response gene induction. Individual cytokines that are induced by microbial components triggering Toll-like receptors (TLRs) were reported to be inhibited by HDAC inhibitors.[3-5] Yet, the extent of those inhibitory effects and possible functional consequences during infections were largely unknown.

In this issue, Roger et al used genome-wide expression profiling to study global alteration of TLR-induced gene induction by HDACs in macrophages.[6] Surprisingly, up to 60% of genes transcriptionally increased by TLR2 or TLR4 stimulation were inhibited in the presence of the broad-range HDAC inhibitor trichostatin A, whereas only 16% of genes were potentiated. Gene activity that was inhibited included all major functions of activated macrophages, such as microbial sensing by pattern-recognition receptors, signal-transduction mediators, transcription regulators, cytokines, chemokines, growth factors, and costimulatory molecules. Thus, this work unexpectedly found that HDAC inhibitors were mostly immunosuppressive.

Of note, these results find their counterpart in vivo: treatment of mice with the HDAC inhibitor valproate increased the susceptibility to develop pneumonia by *Klebsiella pneumoniae* or systemic candidiasis. Conversely, HDAC inhibition conferred protection in models of septic shock by limiting the cytokine burst. Corroborating these observations, it has been reported previously that patients treated with HDAC inhibitors show an increased susceptibility to develop severe infection even without neutropenia.[7] Inhibiting cytokine activity is also known to affect microbial susceptibility in studies using tumor necrosis factor (TNF) inhibition. Thus, within the ongoing HDAC inhibitor trials attention should be paid to infectious susceptibility. The protective effect of HDAC inhibitors on dysregulated inflammation offers a potential therapeutic intervention in the treatment of sepsis. Of note, and taking into account the link between chronic inflammation and cancer, one might wonder whether part of the therapeutic activity of HDAC inhibitors in cancer therapies is due to the pronounced inhibition of inflammation.[8]

Defining the mode of action of HDAC inhibitors on the immune system is clearly needed, yet so far only poorly understood. The article by Roger et al suggests a novel activity of HDAC inhibitors, that is induction of the chromatin modifier Mi-2β. Enhanced promoter recruitment of Mi-2β to the interleukin 6 (IL-6) promoter is followed by inhibition of IL-6 transcription. Mi-2 is part of the nucleosome remodeling, histone deacetylation (NuRD) complex. Mi-2β/NuRD acts antagonistically to the SWI/SNF nucleosome-