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Microparticles as Autoantigens: Making Immune Complexes Big

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The paper by Nielsen et al in this issue of *A&R* makes a simple but important point: immune complexes can represent very big structures whose dimensions dwarf the small size expected for the usual pairings of antibody and antigen in solution (1). Indeed, as these studies show, immune complexes in the plasma of patients with systemic lupus erythematosus (SLE) include sub-cellular structures called microparticles (MPs) whose surface is studded with IgG, IgM and C1q, the hallmarks of antibody deposition and complement activation. Furthermore, these studies demonstrated that the number of MPs bearing IgG as well as the density of IgG on particles are increased in lupus even though the total number of MPs is not elevated. By focusing on the structural components of immune complexes in lupus, this study provides a new perspective for understanding the mechanisms driving autoantibody production in SLE and the role of immune complexes in immunopathogenesis.

Interest in the role of MPs in pathogenesis of immune-mediated and vascular disease is now surging although investigation of these structures extends back several decades. As shown by a variety of analytic approaches, MPs are small membrane-bound vesicles that are released from cells undergoing activation or death. As they are released, MPs carry along with them normal cellular constituents including molecules from the membrane, cytoplasm and even the nucleus. Particles range in size from about 0.1 to 1.0 microns and are usually measured by flow cytometry. This technique can detect particles by light scatter or, using approaches analogous for enumerating cells, immunofluorescent probes for surface markers. Since ordinary cytometers are limited in the detection of objects below 0.1 to 0.2 microns in diameter, determining the full range of particle size and number in a specimen can be difficult (2,3).

While particles can emerge from cells during cell death, they differ from apoptotic bodies which are the large fragments or collapsed remnants of the dying cells. In addition to their size, MPs differ from apoptotic bodies in the time course of their production. Apoptotic bodies are end-products of programmed cell death, a corpse in other words. In contrast, MP release can be a feature of early apoptosis (2).

The first MPs defined in blood came from platelets and were termed platelet dust. While these particles were originally considered inert, subsequent studies demonstrated important pro-thrombotic activity, most likely related to the expression of tissue factor and phosphatidylserine (PS) at the membrane surface, Surface expression of PS results membrane “flipping” and promotes coagulation. The number of platelet particles is

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frequently increased in the blood during diseases with a strong vascular component such as stroke, atherosclerosis and diabetes. Particles can therefore provide a useful biomarker in these conditions (4).

As indicated by *in vitro* studies of particle generation and phenotyping of blood MPs, most if not all cells can produce MPs during either activation or death. Furthermore, studies have shown that, in addition to promoting thrombosis, MPs have important immunological activities and can act, in intercellular communication, to stimulate many cell types including immune cells, fibroblasts and endothelium among others. Indeed, particles can serve as highly potent immune stimulants with their activity likely resulting from their content of various immunoactive molecules, including cytokines such as IL-1 (2,3,5).

In the context of the study by Nielsen et al, the immunological significance of the particles relates to their ability to bind autoantibodies rather than either their pro-thrombotic or pro-inflammatory activity. Thus, as the data presented indicate, blood from patients with SLE have dramatically increased levels of MPs bearing surface IgG, IgM and C1q whose numbers and density are greater than compared to those of normal controls or patients with other diseases. Nevertheless, total particle numbers measured by flow cytometry were actually lower in the blood of patients than those found in other disease as shown in previous studies from the same investigators (6). The findings differ from studies by others indicating increased numbers of particles of various types in lupus (7–9); possible explanations relate to methodology and the measurement by Nielsen et al of only particles that are annexin V positive, indicative of surface expression of PS. An aspect of methodology that can impact on particle measurement relates to sample preparation, including freezing and thawing prior to flow cytometry as was done in this study. While these methodologic issues may affect determination of particle number and size, the important results flow cytometry in the current study were confirmed by mass spectrometry which provided decisive evidence for the presence of IgG, IgM and C1q in the particles.

While the current studies do not address the mechanisms by which immune reactants appear on MPs, the correlation with the presence of antibodies to DNA, ENA and histones suggests direct antibody binding, likely related display of nuclear autoantigens by MPs arising during apoptosis. As now recognized, apoptosis is an enormously dynamic and elaborate process in which the cell essentially rearranges and disassembles itself before its final demise. During this process, nuclear molecules translocate from the nucleus into structures called blebs (10). Since blebbing and particle release occur concurrently during apoptosis, MPs are likely blebs that detach from cells.

These considerations put the bleb at center stage during the generation of self-antigen driving autoimmunity. This role may be especially prominent in lupus where the number of dead and dying cells may rise because of either accelerated apoptosis or a deficiency in the humoral or cellular mechanisms that clear away dead cells before they release constituents that drive inflammation (11). As shown using various microscopic techniques, a bleb is a balloon or bubble-like structure that protrudes from the surface of cells undergoing apoptosis (12,13). The function of blebbing is not fully understood although blebbing may regulate intracellular tension as the surface to volume ratio of dying cells changes during apoptotic shrinkage. As it forms, the bleb incorporates its surroundings, providing a neat package for export into the extracellular space in a process that has striking resemblance to the release of virus particles. Indeed, there has been speculation that viruses have hijacked a normal cellular process for more devious purposes.

As shown in pioneering work by Casciola-Rosen, Rosen and their colleagues, nuclear molecules that serve as lupus autoantigens are prominent bleb components. Indeed, location

in blebs, along with proteolytic cleavage that autoantigens may undergo during apoptosis, has been posited as an important explanation for the immunogenicity of certain self-molecules (10,14). Blebbing in particular may enhance immunogenicity of nuclear molecules by causing greater exposure to the immune system or disposition in a form that enhances presentation when taken up by antigenic presenting cells.

The original studies on the disposition of nuclear antigens focused on blebs when attached to cells. If MPs are detached blebs, then nuclear molecules can readily enter the circulation albeit as a particle rather than as isolated or biochemically discrete entities. Indeed, studies by Ullal et al demonstrated that MPs obtained from the cultures of apoptotic cells can serve as autoantigens for lupus autoantibodies (15). Using flow cytometry, these studies showed that some but not all murine monoclonal anti-nucleosome antibodies, including an anti-DNA antibody, bound to the particles; in the case of this anti-DNA, antibody binding depended on DNA since DNase digestion reduced the level of antibody binding. Similarly, plasma from patients bound to particles generated *in vitro.* A simple correlation of particle binding with anti-DNA levels was not found, however, likely reflecting the serological heterogeneity of patient sera and the ability of autoantibodies of a variety of specificities to bind to particles. As in the case of the study by Nielsen et al, Ullal et al showed that patient plasma had particles with IgG although the presence of IgM and C1q was not tested.

The ability of antinuclear antibodies to bind to MPs is perhaps not surprising since MPs have a rich supply of DNA and RNA and other nuclear molecules such as histones. For these nuclear components to be antigenic and form immune complexes, however, they must be an accessible to antibody. This accessibility could relate to either surface display of the autoantigen or an open particle structure that allows antibody penetration into the particle interior. Since particles originate from apoptotic cells which have been undergone membrane permeability changes, their membranes are likely sufficiently porous to allow ingress of antibodies as well as other serum proteins.

The persistence of DNA in particles nevertheless must be reconciled with the potential for digestion by serum nucleases which should also have access to DNA whether on the surface or particle interior. In that situation, nuclease digestion could remove antigenic DNA although it is possible that particle DNA is at least partially protected from digestion in the confines of an MP, leaving some intact and able to bind antibody. It is interesting to note therefore that, in the study by Ullal et al, one of the anti-DNA antibodies tested failed to bind particles, perhaps related to more prominent display of certain epitopes or elimination of others by digestion (15).

The binding of antibodies and complement to circulating particles may reflect the classical assembly of an immune complex, albeit with a big “antigen,” although other explanations are possible. Thus, particles, because of their expession of Fc or complement receptors, may bind pre-assembled complexes formed in the circulation from either nuclear or other antigens. Another possibility for the presence of immune reactants on MPs is that antibody binding and fixation of complement occurred with antigens on the cell surface, with that structure ejected as part of a cell-protective mechanism to eliminate the pores formed from assembly of membrane attack complexes. Indeed, there is evidence that the cell employs a number of strategies to defend itself against membrane injury including patching or popping out dangerous leaks (16).

Whatever the origin of the particles with immunoglobulin and complement, they can serve as novel elements in disease pathogenesis either by depositing in the kidney to incite nephritis or stimulating plasmacytoid dendritic cells to produce type 1 interferon. There are many potential differences between a small immune complex comprised of a soluble

molecule compared to a large complex comprised of particles. Beyond size, these differences include mechanisms of clearance, interaction with serum proteins and the existence of an extended surface for simultaneous receptor stimulation with an array of immunoactive molecules; these molecules include cytokines as well as ligands of the toll-like receptors. In this regard, investigators have used supernatants of apoptotic cells as a source of nuclear antigens to form immune complexes to stimulate interferon production in *in vitro* models systems. Perhaps such supernatants had MPs as a secret ingredient to generate responses (17).

As studies in cell biology have shown, the cell is not just a solution of proteins and nucleic acids. Rather, it is a highly organized structure in which the macromolecule components exist in tight and intimate relationships with each other, forming a rich gel in which the different elements are interconnected and interwoven in regions. Nevertheless, immunologists trying to define the antigenicity of self-molecules have focused on purified and discrete antigens. While this focus likely reflects the heritage of immunologists in creating vaccines and using model systems with simple protein antigens, it neglects the fact that, in cells, molecules are neither “pure” nor discrete but exist in ensemble that can include particulates, whether intra or extracellular.

The study by Nielsen is a strong reminder that immune complexes are at the heart of key events in lupus and understanding these events may involve studies with messy, complicated and seemingly ill-defined structures such as particles. While concentrating on such crude material may dismay the immunochemists among the lupologists, it should revive interest in the detailed process of immune complex assembly. It should also represent a welcome innovation for investigators searching for new biomarkers or trying to elucidate the mystery of self-antigen recognition. Hopefully, future studies will define the biology of particles and help answer the important question of whether, in understanding lupus, particles represent something big or something small.

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References

1. Nielsen CT, Ostergaard O, Stener L, Iversen LV, Truedsson L, Birgitta G, et al. Increased IgG on cell-derived plasma microparticles in systemic lupus erythematosus is associated with autoantibodies and complement activation. Arthritis Rheum. 2011 submitted.
2. Beyer C, Pisetsky DS. The role of microparticles in the pathogenesis of rheumatic diseases. Nat Rev Rheumatol. 2010; 6:21–29. [PubMed: 19949432]
3. Gyorgy B, Szabo TG, Pasztoi M, Pal Z, Misjak P, Aradi B, et al. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. Cell Mol Life Sci. 2011; 68:2667–2688. [PubMed: 21560073]
4. Rautou PF, Vion A-C, Amabile N, Chironi G, Simon A, Tedgui A, et al. Microparticles, vascular function, and artherothrombosis. Circ Res. 2011; 109:593–606. [PubMed: 21852557]
5. Boilard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. Science. 2010; 327:580–583. [PubMed: 20110505]
6. Nielsen CT, Østergaard O, Johnsen C, Jacobsen S, Heegaard NH. Distinct features of circulating microparticles and their relationship to clinical manifestations in systemic lupus erythematosus. Arthritis Rheum. 2011; 63:3067–3077. [PubMed: 21702008]
7. Sellam J, Proulle V, Jüngel A, Ittah M, Miceli RC, Gottenberg JE, et al. Increased levels of circulating microparticles in primary Sjögren’s syndrome, systemic lupus erythematosus and

rheumatoid arthritis and relation with disease activity. Arthritis Res Ther. 2009; 11:R156. [PubMed: 19832990]

8. Duval A, Helley D, Capron L, Youinou P, Renaudineau Y, Dubucoquoi S, et al. Endothelial dysfunction in systemic lupus patients with low disease activity: evaluation by quantification and characterization of circulating endothelial microparticles, role of anti-endothelial cell antibodies. Rheumatology (Oxford). 2010; 49:1049–1055. [PubMed: 20211868]
9. Oyabu C, Morinobu A, Sugiyama D, Saegusa J, Tanaka S, Morinobu S, et al. Plasma platelet-derived microparticles in patients with connective tissue diseases. J Rheumatol. 2011; 38:680–684. [PubMed: 21239749]
10. Casciola-Rosen LA, Anhalt G, Rosen A. Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. J Exp Med. 1994; 179:1317–1330. [PubMed: 7511686]
11. Muñoz LE, Lauber K, Schiller M, Manfredi AA, Hermann M. The role of defective clearance of apoptotic cells in systemic autoimmunity. Nat Rev Rheumatol. 2010; 6:280–289. [PubMed: 20431553]
12. Charras GT. A short history of blebbing. J Microsc. 2008; 231:466–478. [PubMed: 18755002]
13. Bovellan M, Fritzsche M, Stevens C, Charras G. Death-associated protein kinase (DAPK) and signal transduction: blebbing in programmed cell death. FEBS J. 2009; 277:58–65. [PubMed: 19878312]
14. Rosen A, Casciola-Rosen L. Autoantigens in systemic autoimmunity: critical partner in pathogenesis. J Intern Med. 2009; 265:625–631. [PubMed: 19493056]
15. Ullal AJ, Reich CF 3rd, Clowse M, Criscione-Schreiber LG, Tochacek M, Monestier M, et al. Microparticles as antigenic targets of antibodies to DNA and nucleosomes in system lupus erythematosus. J Autoimmun. 2011; 36:173–180. [PubMed: 21376534]
16. Draeger A, Monastyrskava K, Bablychuk EB. Plasma membrane repair and cellular damage control: the annexin survival kit. Biochem Pharmacol. 2011; 81:703–712. [PubMed: 21219882]
17. Lövgren T, Eloranta ML, Bäve U, Alm GV, Rönnblom L. 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. 2004; 50:1861–1872. [PubMed: 15188363]