ELSEVIER
Nanomedicine: Nanotechnology, Biology, and Medicine
xx (2015) xxx–xxx
nanomedicine
Nanotechnology, Biology, and Medicine
nanomedjournal.com

Platelet mimicry: The emperor's new clothes?

In a recent paper published in *Nature*,[1] Zhang and colleagues used surface platelet mimicry to engineer immune system evading poly(lactic-co-glycolic acid) (PLGA) nanoparticles, and to exploit the acquired platelet-mimicking functionalities for *in vivo* repair of damaged blood vessels, and treatment of systemic infections caused by opportunistic pathogens such as MRSA252, a strain of methicillin-resistant *Staphylococcus aureus*. This approach is further discussed in a *News & Views* commentary in *Nature*.[2]

Cloaking nanoparticles with plasma membranes of erythrocytes and leukocytes was demonstrated earlier.[3,4] The former approach was also developed by Zhang's group.[3] Zhang's current work[1] is a new addition to cloaking approach with biomembrane, but in our opinion, platelet mimicry did not truly confer protection to PLGA nanoparticles against immune recognition, and the reported therapeutic and beneficial effects may have apparently, and predominantly, been related to immune cell (macrophage) interception of the engineered PLGA nanoparticles. Accordingly, the aforementioned processes are similar to what has been reported with traditional drug-loaded nanoparticulate systems for treatment of macrophage infections and other pathologies where macrophages play a critical role in disease progression.[5]

The rational for immune escape

The platelet membrane is enriched with a large number of immunomodulatory glycoproteins including C1-inhibitor, CD55, CD59 and CD47.[6,7] Both CD55 and CD59 are membrane-bound complement regulators, thereby preventing complement attack.[6,7] Indeed, the complement system is the first line of the body's defense against particulate invaders. Complement activation and fixation is a key process for efficient recognition and clearance of particulate invaders by phagocytic cells.[8,9] The third component of the complement system (C3) is responsible for opsonization. Its first cleavage product, C3b, acts as an opsonin and becomes covalently bound to the surface of activating particles; this facilitates particle binding to phagocytes via the complement receptor (CR)1. C3b is further degraded to iC3b, C3c and C3dg, products that serve as ligands for other complement receptors on leukocytes.[8,10] For instance, iC3b is the primary ligand of the integrin CR3 (Mac-1, CD11b/CD18, $\alpha_M\beta_2$) and integrin CR4 (CD11c/CD18, p150,95, $\alpha_x\beta_2$), but CR3 is the predominant receptor for phagocytic recognition and safer elimination of complement-opsonized particles and complexes.[8,10]

CD47 is a cell surface glycoprotein of the immunoglobulin superfamily, and expressed by virtually all cells in the body.[11] CD47 is further considered as a marker of "self", which regulates phagocytosis through signal regulatory protein alpha in the monocyte/macrophage lineage.[12,13] Indeed, earlier studies have shown that fresh erythrocytes from the blood of CD47-deficient mice exhibit markedly reduced survival on transfusion into wild-type recipients, where complete clearance occurs within 24 h, but their lifespan is normal (45–60 days) in CD47-deficient animals.[12] The rapid clearance of CD47-deficient erythrocytes, however, is complement independent, since they are also cleared from the circulation of C3-deficient mice. Similarly, platelets lacking CD47 also exhibit fast clearance on transfusion into wild-type recipients.[13,14] Furthermore, CD47 analogues are also encoded by pathogens such as smallpox and vaccine virus to disable body's normal defenses.[15]

Cloaking nanoparticles with platelet membranes seems a smart strategy in evading complement system activation and C3 opsonization, as well as conferring general resistance against recognition and ingestion at the hand of macrophages in contact with the blood. Could this approach really work, particularly with the view that platelet-derived membranes can also activate complement?[6]

Cloaked nanoparticles and immune surveillance

Zhang and colleagues showed that cloaking PLGA nanoparticles thwarts complement activation, however, on intravenous injection the liver and the spleen sequestered >90% of nanoparticles within 30 min.[1] These are macrophage-rich organs. Indeed, the hepatic Kupffer cells, and the red-pulp and marginal zone macrophages in the spleen are the predominant scavengers that are in direct contact with the blood capable of intercepting blood-borne particles efficiently and rapidly.[5,10,16] Accordingly, it appears that dressing with platelet membranes does not necessarily disguise nanoparticles against macrophage recognition. This approach seems inferior to the established PEGylation technology. For comparison, Doxil® (a regulatory approved PEGylated liposomal doxorubicin) exhibit a biphasic circulation half-life of 84 min and 46 h in humans.[17] At first instance, Zhang's strategy suggests that thwarting complement

Competing interests: the authors declare no competing financial interest.

activation, and hence C3 opsonization, may not necessarily confer protection to nanoparticles against recognition by professional phagocytes. Phagocytic cells display a battery of pattern recognition receptors that sense particulate invaders and self-effete materials through a wide range of opsonic (also other than C3) and non-opsonic processes, and these may have participated in rapid extraction of cloaked nanoparticles from the blood.[5] Presumably, procedures used for platelet membrane extraction and nanoparticle coating steps have inflicted sufficient membrane damage readily sensed by phagocytic cells. Likewise, the rigidity and the spherical shape of PLGA nanoparticles may have affected the morphology and functionality of the membrane cloak, thus triggering immediate macrophage recognition and clearance.

These above notions are further supported by the authors own observations that antibiotic loaded cloaked nanoparticles were capable of effectively reducing MRSA252 count in both liver and spleen,[1] since macrophages in these organs are home to the pathogen.[18] Zhang and colleagues provide no direct proof that their engineered particles have either intercepted MRSA252 in the blood, where this interaction has resulted in accelerated liver and spleen macrophage sequestration, or encased the bacterium on the macrophage (Kupffer cell) surface. After all, RBC membrane-cloaked nanoparticles, which showed poor tendency to pathogen binding in vitro, had similar therapeutic efficacy to platelet membrane-cloaked nanoparticles. However, with the latter more significant elimination of pathogens in the liver and spleen occurred. This is perhaps due to the fact that RBC membrane cloaking is more effective in conferring resistance against macrophage recognition.[3] Considering the very short circulation times of platelet membrane-cloaked nanoparticles, the reported antimicrobial efficacy is predominantly the result of direct nanoparticle clearance by tissue macrophage. Indeed, nanoparticulate carriers have long been used for antimicrobial delivery to the infected liver and spleen macrophages; a concept introduced by pioneers such as Alving[19] and Schiffleres and Bakker-Woudenberg.[20] On macrophage internalization, nanoparticles localize to lysosomes.[5] Lysosomal localization brings nanoparticles into close proximity of MRSA252, resulting in their death. Surprisingly, the effect of uncoated antibiotic-loaded PLGA nanoparticles was not compared with cloaked nanoparticles in reducing the hepatic and splenic pathogen load, since bare PLGA nanoparticles instantly target liver and spleen on intravenous injection.[5]

Closer inspection of Zhang's[1] data indicates certain problems with the complement assay in human plasma. C4d, a fluid-phase split product of C4, which is released by complement control protein C4bp and factor I, is a marker of both classical and lectin pathways of the complement system.[21,22] Surprisingly, the background level of C4d in the tested plasma is far below the normal physiological levels[1]; the normal physiological level of C4d is approximately 9 μg/mL plasma. Likewise, measurements of Bb (another complement activation split-product, and a marker of the alternative pathway turnover) are not convincing either and particularly with respect to low responses observed with the positive control zymosan,[1] which is an established potent trigger of the alternative pathway. The causes for these inconsistencies remain unclear and may have been related to blood handling procedures, anticoagulant type, etc. Collectively, these make the interpretation of complement data difficult, and more importantly in the absence of other appropriate controls such as aggregated antibodies and mannan for assessing functionality of the classical and lectin pathways, respectively. For a comprehensive approach, activation of the terminal pathway as well as surface events (e.g., deposition of C1q and opsonic C3 fragments) are still required.

We further emphasize that platelets do express binding sites for classical components of the complement system, most notably for C1q.[23] Indeed, C1q interactions with platelets are known to trigger responses that may contribute to inflammation and thrombosis.[23,24] The expression of C1q binding sites on human platelets together with the ability of gC1qR to engage the globular domain of C1q indicates that platelets may have an intrinsic ability to initiate the classical complement pathway.[24] Accordingly, translational aspects of nanoparticle cloaking with cell membranes should not be viewed as trivial, since minor damages may yield inconsistent preparations capable of triggering immune responses.

Other acquired platelet-mimicking functionalities

The cloaked nanoparticles, however, exhibited certain platelet-mimicking functionalities.[1] These have included in vitro pathogen binding (since MRSA252 is a platelet-adhering pathogen), collagen adhesion, and adherence to denuded artery. In a rat model of coronary restenosis, the cloaked nanoparticles showed some therapeutic efficacy on docetaxel loading, and improved the damage,[1] but this efficacy, although not evaluated, may again be related to nanoparticle disposition to the local immune cells (pathological macrophages).[5] It should be emphasized that absolute nanoparticle targeting is still marginal, and corresponds to a minor fraction of the injected dose.[1] A slight selectivity in organ uptake (and an acute pharmacological effect) should not be heralded as 'targeting' and 'therapeutic success' even when a few percent of the administered dose reaches the desired site. This poses a question concerning the fate of unaccounted material, which in the present case includes liver and spleen,[1] and hence the overall safety and in relation to multiple dosing and the overall therapeutic regimens. The significance of Zhang's[1] approach compared with more traditional targeting strategies employing ligand-decorated nanoparticles, however, warrants future evaluation. Indeed, there are many examples of simpler ligand-decorated nanoparticles that target damaged vessels, and suggested in experimental imaging and therapeutic interventions.[25,26] Again, with these systems, a minor fraction of the injected dose reaches the pathological target.

Shape

It should be emphasized that platelet functions are also modulated by their shape, and cell flexibility/deformability.[27] Indeed, platelets possess the inherent ability to marginate to vascular wall and specifically interact with injured sites. Platelet membrane-cloaked nanoparticles, or platelet-derived engineered vesicles, may yet realize this potential through precision integrated shape,