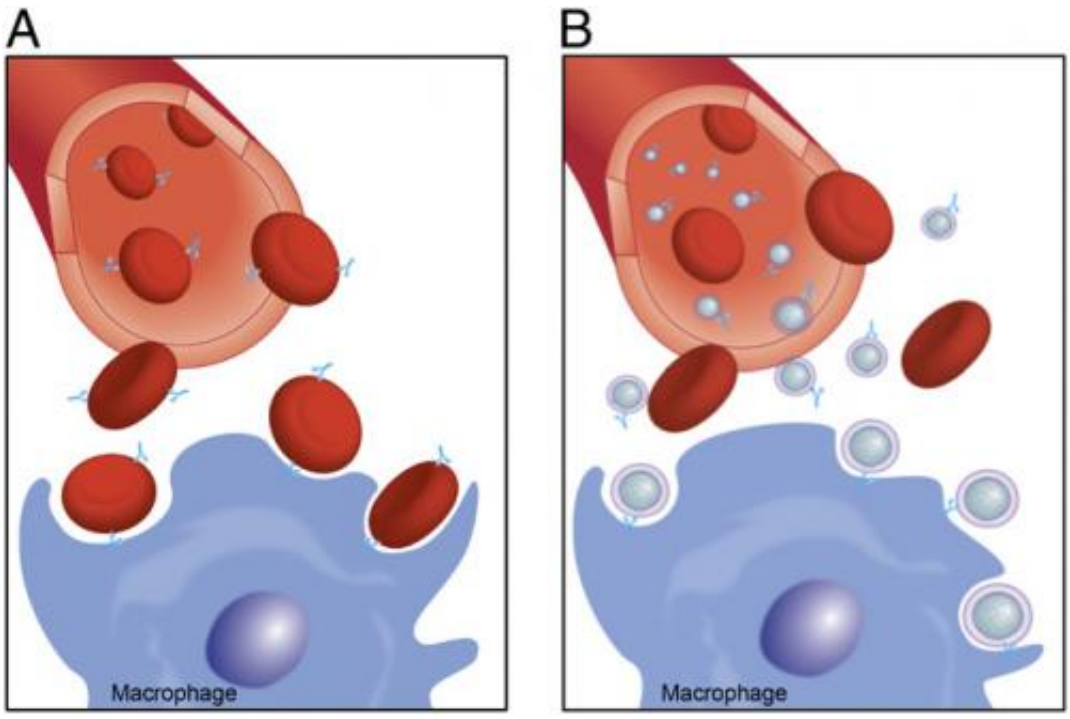
Diminutive decoys: Membrane-cloaked nanoparticles disrupt antibody-mediated autoimmune diseases

September 19 2014, by Stuart Mason Dambrot



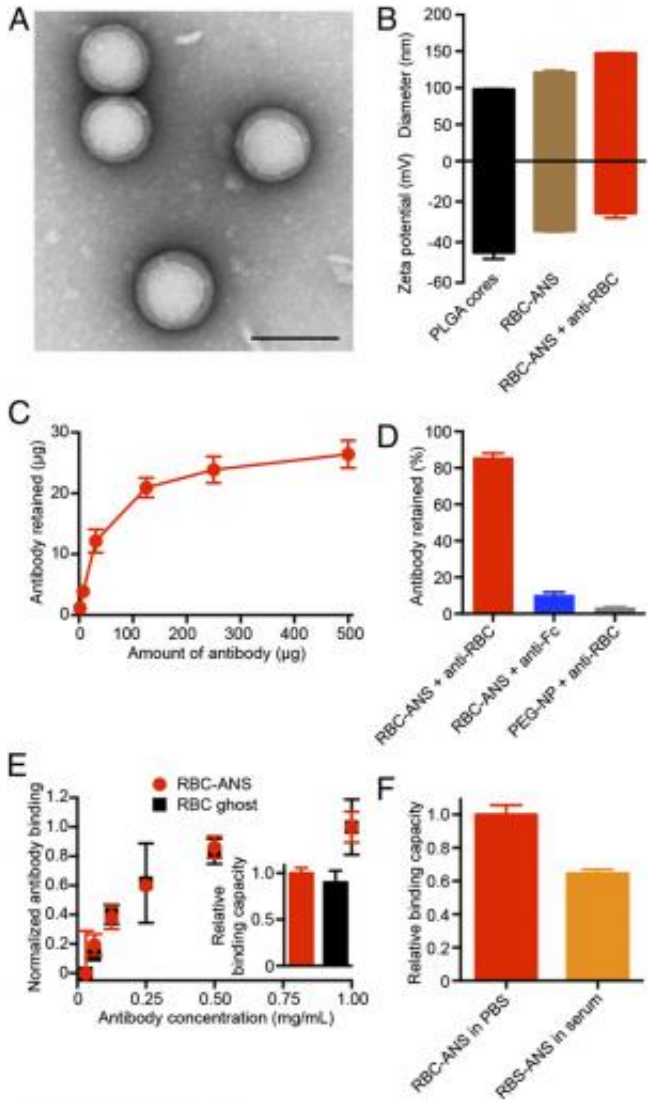
Schematic representation of RBC-ANS neutralizing anti-RBC antibodies (anti-RBCs). (A) Anti-RBCs opsonize RBCs for extravascular hemolysis, via phagocytosis, as observed in AIHA and DIA. (B) RBC-ANS absorbs and neutralizes anti-RBCs, thereby protecting RBCs from phagocytosis. Credit: Copp JA, et al. (2014) Clearance of pathological antibodies using biomimetic nanoparticles. *Proc Natl Acad Sci* USA 111(37): 13481-13486.

(Medical Xpress)—What do rheumatoid arthritis, type I diabetes, myasthenia gravis, multiple sclerosis, rheumatic heart disease, and narcolepsy have in common? All of these (and many other) apparently unrelated disorders are caused by *autoimmunity*, in which the immune system produces antibodies that attack normal, healthy cells and tissues. Currently considered incurable, these autoimmune diseases can be managed – albeit with varying efficacy and sometimes serious side effects – by immunosuppressive (reducing the activation or efficacy of the immune system), anti-inflammatory (steroids), or palliative (for example, insulin injections if type 1 diabetes) treatment. Moreover, autoimmune diseases include a wide range of dysfunctional immune responses known as type II, type III, and type IV immune hypersensitivity reactions.

Recently, scientists at University of California, San Diego demonstrated a novel pathophysiologically-inspired nanoengineering approach to the management of type II hypersensitivity reactions in which antibodies produced by the immune response bind to intrinsic or extrinsic antigens on the surfaces of healthy cells, such as a potentially fatal reaction to penicillin. (Antigens, typically microorganisms and chemicals, provoke an immune response and, if foreign or toxic, bind to a specific antibody.) The researchers demonstrated in a murine model of antibody-induced anemia that by successfully acting as alternative targets for anti-RBC antibodies, polymeric nanoparticles coated with intact red blood cell (RBC) membranes – which the scientists term *RBC antibody nanosponges* (ANS) – counteracted the effect of pathological antibodies without requiring pharmaceutical immune suppression and thereby protected circulating healthy RBCs. The nanosponges reduced antibody binding to healthy RBCs by up to 95% in a test tube study, and mice injected with anemia-inducing antibodies followed by injection with the nanosponges showed improvements in anemia-related parameters compared with mice injected with antibodies and nanoparticles *not* coated with an RBC membrane.

Prof. Liangfang Zhang discussed the paper that he, Jonathan A. Copp and their co-authors published in *Proceedings of the National Academy of Sciences*. "In type II immune hypersensitivity, the antibodies produced by the immune system bind to the antigens on the patients' own cells, thus causing the cells to be destroyed," Zhang tells *Medical Xpress*. . "While current treatment is largely relying on broadly suppressing the immune system, which is non-specific and likely to induce adverse side effects, RBC nanosponges serve as an ideal decoy to absorb these pathological antibodies and thus divert them away from the cellular targets." In so doing, the RBC nanosponges can deplete the level of circulating antibodies, and so save natural RBCs without introducing any therapeutic drugs – and more importantly, Zhang adds, these particles themselves are completely biocompatible and biodegradable, so they can be broken down to small molecules and leave nothing that is harmful to the body.

In addition, the team has developed an established protocol to fabricate these biomimetic nanoparticles consisting of synthetic polymeric cores surrounding by natural RBC membranes: the core ensures mechanical stability by supporting the RBC membrane shell, while membrane provides an ideal stealth coating that evades immune recognition. "Nanoparticles have a size similar to that of a virus, so without appropriate protection can be quickly cleaned from the bloodstream," Zhang notes. "Since the major challenge to achieving long circulation is attack by the immune system, this stealth feature allows the nanoparticles to circulate in the bloodstream for longer periods of time, which has significant clinical impact in sustained systemic cargo delivery or toxicant clearance." In the present study, the longer the nanosponges circulate in the bloodstream, the greater their opportunity to absorb pathological antibodies.



In vitro characterization of RBC-ANS. (A) TEM image demonstrates the core/shell structure of RBC-ANS. (Scale bar: 150 nm.) (B) Size and surface zeta-potential of pure PLGA cores, RBC-ANS, and RBC-ANS bound with anti-RBCs. (C) RBC-ANS (250 μg) incubated with five serial dilutions of fluorescent anti-RBCs demonstrated particle saturation at ~27 μg of antibody, corresponding to a particle/antibody mass ratio of ~9:1. (D) Equivalent amounts of RBC-ANS incubated with anti-RBCs or anti-Fc demonstrated significantly greater specific binding compared with nonspecific binding. The corresponding PEG-NP incubated with anti-RBCs served as a negative control. (E) Comparison of anti-RBC binding kinetics with a fixed amount of RBC-ANS or RBC ghosts. (Inset) Relative binding capacity of RBC-ANS vs. RBC ghosts at saturation. (F) Relative binding capacity of RBC-ANS in PBS vs. RBC-ANS in serum at

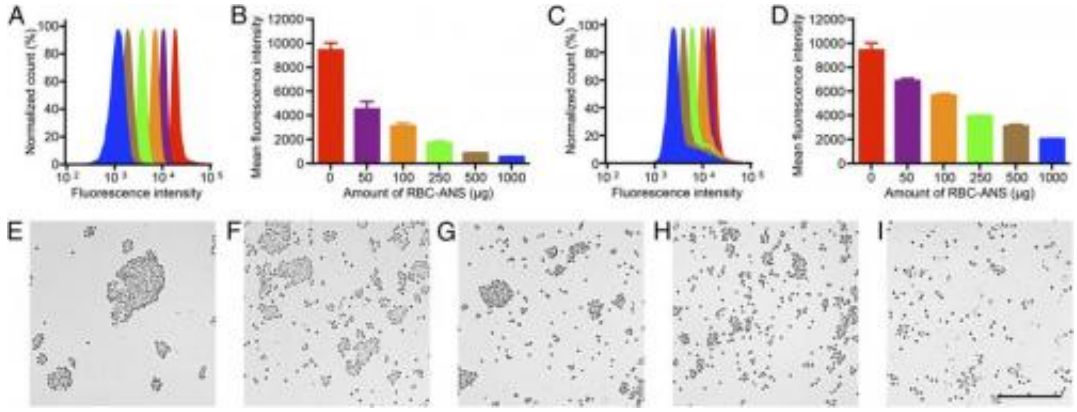
saturation. Credit: Copp JA, et al. (2014) Clearance of pathological antibodies using biomimetic nanoparticles. *Proc Natl Acad Sci* USA 111(37): 13481-13486.

That said, Zhang points out that a major challenge of using the nanosponges to absorb pathological antibodies is to determine how many antibody nanosponges – that is, the dosage – are needed in order to remove sufficient amount of the antibodies and thus to reduce symptoms. "This requires us to do a careful dosing study on the nanosponges' absorption capability."

While the scientists were inspired by antibody-driven pathology in general, Zhang tells *Medical Xpress* that they are particularly interested in type II immune hypersensitivity, where the antibodies produced by the immune system bind to the antigens on the patient's own cells and thereby cause the cells' destruction. "The key innovation was to create a biomimetic nanoparticle that serves as an antibody decoy to absorb and arrest the pathological antibodies that otherwise will bind to natural cells and destroy them," he says. "These biomimetic nanoparticles are about 120 nm in size, circulate for days in the blood stream to absorb pathological antibodies, and are eventually broken down safely by the liver, leaving nothing toxic – a therapeutic approach expected to be much safer and cheaper than existing treatments."

Moreover, it turns out that the nanosponges can be prepared relatively easily. "In order to evade the immune system and remain in circulation, the biomimetic nanoparticles are wrapped in red blood cell membranes – a fabrication process is rather straightforward," Zhang explains. "We collect cell membranes from natural cells and fashion them to small vesicle form; prepare polymeric cores; and merge the vesicle and core together. Finally, the cell membrane-derived vesicles will spontaneously fuse onto the polymeric core under vortexing or sonication (agitating the

particles by applying ultrasonic sound), resulting in biomimetic nanosponges." This simple fabrication process allows for large-scale nanoparticle manufacturing.



In vitro dose-dependent neutralization and stability of RBC-ANS/anti-RBC binding. (A) Flow cytometry histograms of RBC-ANS (from left to right: 1,000 μg, 500 μg, 250 μg, 100 μg, 50 μg, and 0 μg) preincubated with 50 μg of FITC–anti-RBCs before mixing with 5 vol% RBC solution demonstrated dose-dependent neutralization of anti-RBCs. (B) Mean fluorescence intensity of samples in A. (C) Flow cytometry histograms of RBC-ANS (from left to right: 1,000 μg, 500 μg, 250 μg, 100 μg, 50 μg, and 0 μg) coincubated with 50 μg of FITC–anti-RBCs and 5 vol% RBC solution demonstrated dose-dependent, competitive neutralization of anti-RBCs. (D) Mean fluorescence intensity of samples in C. (E–I) Varying amounts of RBC-ANS (from E to I: 0 μg, 25 μg, 50 μg,100 μg, and 250 μg) were coincubated with 15.6 μg of anti-RBCs (primary antibody) and 5 vol% RBC solution, followed by adding an equivalent dose of anti-Fc (agglutinating secondary antibody). The samples were then imaged by light microscopy at 10× magnification, demonstrating dose-related inhibition of RBC agglutination by RBC-ANS. (Scale bar: 100 μm.) Credit: Copp JA, et al. (2014) Clearance of pathological antibodies using biomimetic nanoparticles. *Proc Natl Acad Sci* USA 111(37): 13481-13486.

In addition to how nanosponges clear pathological antibodies from the

bloodstream in the RBC protocol discussed in the paper, Zhang points out that for other types of type II immune hypersensitivities it depends on the cell types the autoantibodies target. "The reported RBC nanosponges can be applied to clear all autoantibodies that target RBCs. If they target other types of cells, we can use those cells to fabricate the types of nanosponges needed to treat those particular type II hypersensitivities."

Moving forward, the scientists are focused on further validation of RBC antibody nanosponges for the treatment of RBC-related type II immune hypersensitivity in different animal models – the goal being human clinical trials – and are investigating other antibody nanosponges fabricated from various cell types. In addition, Zhang adds, they have a few other innovative systems under development right now, with a patent application in process.

"This study certainly advances the development of nanotechnology and bioengineering research by providing a unique and robust nanoparticle platform that combines the strengths of both synthetic nanoparticles and natural cellular membranes," Zhang concludes. "It will therefore also benefit a broad range of biomedicine research by providing a new therapeutic option that offers opportunities for selective disease intervention while minimizing risk and side effects associated with many traditional drug-based therapies."

More information: Clearance of pathological antibodies using biomimetic nanoparticles, *Proceedings of the National Academy of Sciences USA* September 16, 2014 vol. 111 no. 37 13481-13486, doi:10.1073/pnas.1412420111