Variability of opsonization of iron oxide nanoparticles with complement C3 in different species and strains: a quest for a predictive animal model

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Introduction

- The complement system plays a key role in opsonization and immune clearance of engineered nanoparticles.
- A wide range of nanoparticles trigger complement activation in sera of mice, dogs, rats and pigs as well as in vivo following intravenous injection.
- There is a limited understanding whether complement activation by nanomedicines in preclinical species can predict human response.
- Multiple nanoparticle types can be used to study complement activation, including carbon nanotubes, micelles, liposomes, polymeric nanospheres, iron oxide, and gold.
- We studiesmultiple preclinical species and strains using several dextran-coated SPIO, either clinically available, or prepared in our laboratory to understand the differences between humans and animal models in complemen activation, in order to find a better animal model for prediction in human's complement activation.

Method

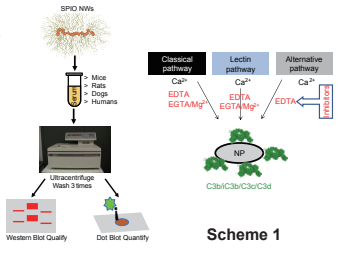
Study Design: Use dextran-coated SPIO nanoparticles to test the complement activation in mice, rats, dogs and humans serum. (Scheme 1)

Nanoparticles: All particle solutions were at 1 mg Fe/mL and had less than 0.2 EU/mL endotoxin as measured with Limulus amebocyte lysate assay. (Table 1)

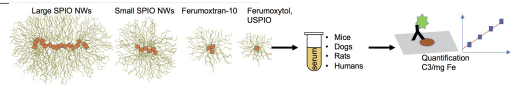
Dot Blot assay: Nanoparticles (1 mg/ml Fe) were added to undiluted serum and incubated for 30 min at 37 ◦C, purified with ultracentrifugation and blotted on a nitrocellulose membrane at 1 µg Fe/dot. C3 was quantified with anti-C3 antibodies against respective species. (Scheme 2)

Particle name	Composition	Size (intensity weighted), nm	PDI	Zeta potential, mV
Large SPIO NW	20 kDa native dextran, multiple crystals per particle	106 (Z-average), 126 ± 54.42 (peak)	0.16	−7.4 ± 6.8
Small SPIO NW	20 kDa native dextran, multiple crystals per particle	67 (Z-average), 81.36 ± 40.35 (peak)	0.22	−6.8 ± 8.8
Ferumoxytol (Feraheme ®)	10 kDa reduced carboxymethyl dextran, 1 crystal per particle	26 (Z-average), 26.86 ± 10.53 (peak)	0.27	−15.6 ± 7.4
Ultrasmall SPIO (USPIO)	10 kDa reduced T-10 dextran SPIO, 1 crystal per particle	26 (Z-average), 25.96 ± 7.64 (peak)	0.23	−5.0 ± 10.0
Ferumoxtran-10	10 kDa native T-10 dextran SPIO, 1–3 crystals per particle	44 (Z-average), 51.19 ± 20.75 (peak)	0.14	−7.2 ± 9.5

Table 1



Scheme 1



Scheme 2

Results

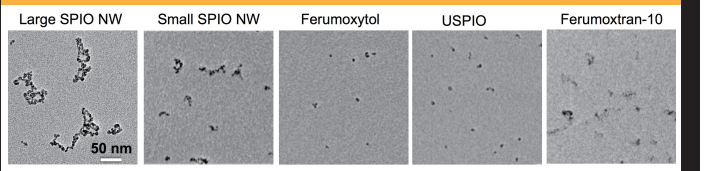


Figure 1. Transmission electron microscopy of the nanoparticles used in the study. Size bar 50 nm for all images.

Results

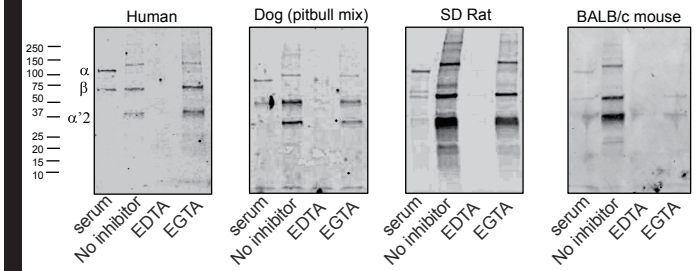


Figure 2. Western blot analysis of native C3 in sera and nanoparticle-deposited C3 in different species. C3 was detected by species-specific anti-C3 antibodies.

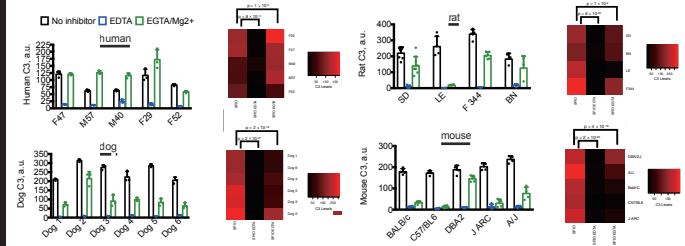


Figure 3. C3 opsonization pathways of SPIO NWs in multiple species and strains. Bar graphs show levels of C3 (raw signal intensity) and heat maps represent C3 levels (relative intensities within species) with statistical significance between means. Each heat map corresponds to the bar graph in the same row.

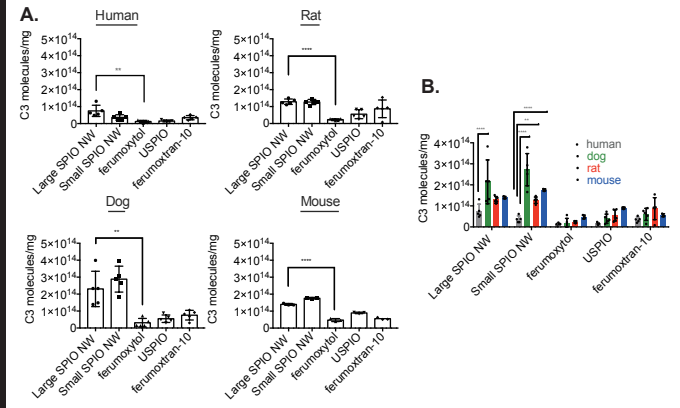


Figure 4. Comparison of C3 opsonization (C3 molecules/mg Fe) of different SPIO formulations across species. A) Mean values of C3 per mg Fe for designated particles, measured in humans (n = 5 donors), dogs (n = 5 breeds), rats (n = 5 strains) and mice (n = 3 strains). B) Comparison of C3 deposition across species (2-way ANOVA with multiple comparisons).

Results

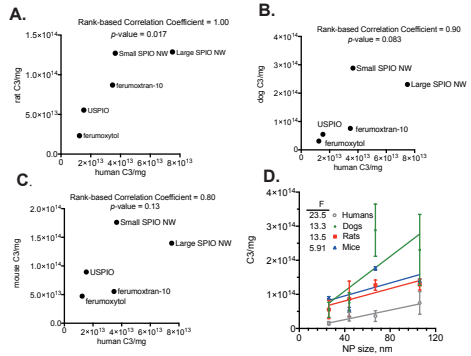


Figure 5. Correlation of C3 deposition on nanoparticles between animals and humans. A) rat vs. human serum; B) dog vs. human serum; C) mouse vs. human serum; D) The number of C3/mg for nanoparticles (excluding ferumoxytol due to charged carboxymethyl dextran) increases with increasing diameter in all species.

Conclusion

- We found significant subject-dependent and strain-dependent differences in the C3 opsonization and conclude that only some of the tested strains display complement response similar to humans.
- There was a strong predictive value of complement opsonization in dog and rat sera; nanoparticles with higher C3 deposition in these species showed higher deposition in humans, and vice versa.
- Notably, the opsonization decreased with decreasing size in all species.
- These studies lay a groundwork for building translational models of complement opsonization of nanomedicines.

Acknowledgement

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References

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