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feature

Mechanism of nanoparticle-induced hypersensitivity in pigs: complement or not complement?

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Q2 **A recent study on nanoparticle-induced hypersensitivity reactions in pigs showed robust pulmonary intravascular macrophage clearance of Polybead® carboxylate microspheres in mediating the adverse Q3 cardiopulmonary distress, irrespective of the ability of these particles to activate the Complement (C) system *in vitro*. Focusing on this observation, this article highlights the controversies in projecting *in vitro* C assay data to *in vivo* conditions and applying data on polystyrene particles to therapeutic nanopharmaceuticals. Based on overwhelming evidence of a role of anaphylatoxins in hypersensitivity reactions, the need to further explore the role of C activation in the reported and other reactions is highlighted. C-activation-related and C-independent pseudoallergies (CARPA and CIPA) can proceed simultaneously, as outlined by the 'double-hit' hypothesis.**

Introduction

Pigs, because of their high sensitivity to nanoparticle (NP)-induced cardiopulmonary distress, have been used as a large animal model for the hypersensitivity reactions (HSRs; see Glossary) observed in humans following intravenous (i.v.) infusion of liposomes and other nanomedicines [1–9]. Specifically, the porcine model imitates the rare (<0.1%) anaphylactic reactions in humans where the hemodynamic and cardiopulmonary distress can lead to cardiac shock and/or death. The above-mentioned earlier studies suggested an association between Complement (C) activation and a tetrad of allergy symptoms involving circulatory, blood cell, cutaneous and biochemical changes, rationalizing the term C-activation-related pseudoallergy (CARPA) [1]. The implication of C activation in the cardiopulmonary distress of pigs is not new – a causal role of anaphylatoxins in HSRs was suggested in the 1980s [10,11]. Since then, multiple studies have provided experimental and clinical evidence supporting the CARPA hypothesis under various conditions (Table 1). The referred porcine studies provided direct (C5a effect, inhibitors) and indirect (correlation) evidence for a role of C activation (Table 1), although direct demonstration of C split products formed during the reactions is still missing.

Regarding the mechanism of CARPA, amplification of the chain reaction by one or more cells of innate immunity was clear from the beginning, with anaphylatoxin receptor expressing mast cells, secretory macrophages, basophils, platelets, and PMN being on the list of potential 'allergomedin' secretory cells [1,12–14]. In the case of the pig model, the probable role of pulmonary intravascular macrophage (PIM) cells was recognized early, owing to the strategic location, C5a receptor (C5aR) positivity and well-known secretory capability of these cells leading to the release of vasoactive histamine, arachidonic acid metabolite eicosanoids (prostaglandins, thromboxanes, leukotrienes) and platelet-activating factor (PAF) [15]. As to the question regarding how these (PIM) cells are activated during NP-induced HSRs, the 'double-hit' theory was proposed in 2012 (Fig. 1) [7], which postulated at least two independent pathways of intracellular signaling – one proceeding via anaphylatoxin receptors and another triggered by direct binding of NPs to different pattern recognition surface receptors. Q4 Q6

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GLOSSARY

ABC phenomenon: accelerated blood clearance of nanoparticles
Anaphylatoxins: C3a, C5a, C activation split products with potent biological activities
Anaphylaxis: the most severe form of hypersensitivity reactions, can be lethal
CARPA: C-activation-related pseudoallergy – pseudoallergy wherein C activation has a role
CIPA: C-independent pseudoallergy
Complement (C): a part of the immune system consisting of ~30 circulating and membrane-bound glycoproteins
Complement (C) activation: cascadic proteolysis of C proteins leading to multiple physiological effects
Hypersensitivity reactions: overreaction of the immune system causing more- or less-severe clinical symptoms
Nanodrugs (nanomedicines, nanopharmaceuticals): complex drugs consisting of nanoparticles
Nanoparticles (NPs): particles in the 10–1000 nm range
PIM cells (pulmonary intravascular macrophages): endothelial-cell-bound macrophages capable of nanoparticle scavenging and secretion of vasoconstrictive mediators
Polystyrene (PS): a synthetic aromatic polymer made of styrene (1-phenylethene)
Pseudoallergy: non-Ig-E-mediated allergy

It was proposed that these pathways can be activated to different degrees at the same time, and that their simultaneous activation might be additive or synergistic in triggering the release of the above-listed vasoactive mediators along with other secretory products (inflammatory cytokines, reactive oxygen species, enzymes, etc.) (Fig. 1). Thus, the double-hit theory embraced and reconciled C-dependent and C-independent pathways of pseudoallergy (CARPA and CIPA) explaining the wide spectrum and substantial inter-species and inter-individual variation of reaction symptoms.

New evidence of complement-independent pseudoallergy (CIPA)

A recent study involving pig experiments in the author's laboratory showed that carboxylated polystyrene NPs (PS-NPs) of spherical shape caused pulmonary hypertension and other hemodynamic changes typical of CARPA; however, these changes could not be correlated with C activation in pig whole blood *in vitro* [16]. Furthermore, there was a correlation between the pulmonary reactivity of spheroidal, prolated and oblated PS-NPs (rods and disks) with their initial clearance from pig and mouse blood, as well as with their uptake by mouse macrophages in culture. In addition, inhibition of macrophages by clodronate liposomes suppressed PS-NP uptake and HSRs. From these data, it was suggested that the pulmonary reaction of pigs to spherical PS-NPs was due to their rapid and robust phagocytosis by PIM cells, irrespective of C activation.

The goal behind revisiting the 'robust phagocytosis' versus C proposal

Unfortunately, in the absence of more space for discussing all aspects of the complex set of *in vitro* and *in vivo* experiments focusing on the impacts of polystyrene particle size, shape and erythrocyte 'hitchhiking' on pulmonary reactivity and macrophage uptake, the *in vitro* data on C activation by different NPs might have been over-interpreted and thus the inactivity of C in the pulmonary reaction overstated [16]. Although at the end of the article it is concluded that the 'exact role of C needs to be explored in detail' [16], to avoid undue generalizations with potential downplaying of an important pathway of HSRs via the well-established C activation route, the author felt it important to revisit the data and extend the discussion of the role of C in the described pulmonary reaction of pigs.

TABLE 1

Clinical and experimental evidence for Complement activation having a causal role in hypersensitivity reactions – excerpts from the literature

Findings	Refs
Human studies	
Anaphylatoxins explain the symptoms of hypersensitivity reaction (HSR) or allergy	[10,11,37–39]
Correlation between strong Complement (C) activation and HSRs to liposomal doxorubicin (Doxil®) in cancer patients	[40]
Correlation between C activation and HSRs to rituximab (Rituxan®) in cancer patients	[41]
C activation was shown to underlie HSRs to Althesin infusion	[42]
Correlation between C activation and cardiac anaphylaxis, the most frequent cause of death in HSRs	[43]
Correlation between C activation and HSRs to dialysis membranes	[44,45]
Correlation between C activation and HSRs to intravenous iron	[46,47]
Large amount of evidence of correlation between C activation and HSRs to radiocontrast agents	[48]
Animal studies	
Correlation between C activation and pulmonary HSRs in various animals	[49]
Administration of human C5a causes cardiopulmonary and hemodynamic changes in pigs mimicking some of the hemodynamic abnormalities of human HSRs	[4]
Complement inhibitors sCR1[a] and IVIG[b] inhibited the cardiopulmonary reaction of pigs to liposomes	[1,50]
Correlation between C activation by liposomes in human serum *in vitro* and HSRs in pigs *in vivo*	[5]

[a] Soluble C receptor type 1.
[b] Intravenous immunoglobulin.

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