

Mini Review

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Rodent models of complement activation-related pseudoallergy: Inducers, symptoms, inhibitors and reaction mechanisms

Abstract: Complement activation-related pseudoallergy (CARPA) is a hypersensitivity reaction to intravenous administration of nanoparticle-containing medicines (nanomedicines). This review focuses on CARPA in rodent models: rats, mice, guinea pigs and rabbits. Information on all aspects of hypersensitivity reactions caused by known complement activators (zymosan, cobra venom factor) and different nanomedicines (liposomes, other drug carrier nanocarriers) in these species has been compiled and analyzed, trying to highlight the similarities and differences. What is most common in all species' reactions to i.v. complement activators, liposomes and other nanoparticles is a dose-dependent hemodynamic and cardiopulmonary disturbance manifested in acute, reversible rise or fall of blood pressure and respiratory distress that can lead to shock. Other symptoms include heart rate changes, leukopenia followed by leukocytosis, thrombocytopenia, hemoconcentration due to fluid extravasation (rise of hematocrit) and rise of plasma thromboxane B₂. The results of a recent rat study are detailed, which show that rats are 2–3 orders of magnitude less sensitive to liposome-induced CARPA than pigs or hypersensitive humans. It is concluded that CARPA can be studied in rodent models, but they do not necessarily mimic the human reactions in terms of symptom spectrum and sensitivity.

Keywords: anaphylaxis; complement; liposomes; pseudoallergy; rats; rodents.

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Introduction

Complement activation-related pseudoallergy (CARPA) is a hypersensitivity reaction to intravenous administration of nanoparticle-containing medicines (nanomedicines) in patients with occasionally serious consequences (1–4). The details of CARPA and its mechanisms are described in other chapters of this Special Issue. New nanomedicines should be tested in animals prior to human administration in order to avoid toxicity, including CARPA. Currently the porcine model of CARPA is the best to predict potential safety concerns of nanomedicines as the pig is highly sensitive to i.v. treatment with liposomes and other nanoparticles, as the resulting physiological changes can be extrapolated to humans with high certainty (5). However, new, less expensive models are also needed to advance the testing of nanomedicine-induced CARPA. Complement (C) activation can be easily tested in vitro, which is an inexpensive and rapid evaluation of potential safety risk (6, 7). Such in vitro tests can be tailored to fulfill species specificity requirements. However, the problem with in vitro-based systems is that the effector arm of immune response, most importantly the cardiovascular system, is not present (8). Therefore, reliable prediction of immunotoxicity requires a battery of tests which includes both in vitro and in vivo models (9). Rodents are especially suitable for this purpose as huge amount of information is available concerning the pathophysiology of C activation in rodent species. However, rodents seem to be much less sensitive to nanomedicines than pigs (10, 11). The aim of the current review is to summarize

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the available information on CARPA in rodents to get an insight into various C activation mechanisms elicited by nanomedicines that may be present in various rodent species.

The CARPagenic effects of CVF, zymosan and LPS in rodents and their modulation with complement antagonists

Cobra venom factor (CVF) is a rapid activator of the complement system, and intravenous treatment of rodents with CVF is a model of acute respiratory distress syndrome, a severe illness due, in part, to C activation. Some of the physiological changes caused by CVF in the presence or absence of C inhibitors include the following observations. Pretreatment of anesthetized rats i.v. or p.o. with AcF-[OP(D-Cha)WR], a C5a receptor (C5aR) antagonist, markedly attenuated CVF-induced (4 IU/kg) drop in polymorphonuclear (PMN) cell count and the long-lasting hypotension, but it did not alter the transient increase in blood pressure. On the other hand, N(2)-[(2,2-diphenylethoxy)acetyl]-L-arginine, a C3a receptor (C3aR) antagonist, caused neutropenia on its own, which was similar to that of caused by CVF. It also attenuated CVF-induced transient hypertension but did not alter hypotension. Inhibition of both C3 and C5 convertases by rosmarinic acid (12) inhibited all the above responses. The increase in pulmonary vascular permeability was inhibited most by the C5aR antagonist. Rosmarinic acid was less effective, and the C3a receptor antagonist was the least effective in this respect. All three antagonists diminished the increases in plasma TNF α levels that peaked at 60 min after CVF administration (13).

A part of endotoxin (LPS) shock can be attributed to C activation as plasma levels of both C3a and C5a are markedly elevated in LPS-treated rats (14). The underlying physiological changes include systemic hypotension and increased hematocrit, along with decreases of the leukocyte (PMN), monocyte, and platelet counts. Prior administration of a rat anti-C5a antibody failed to alter the hematologic changes and pulmonary edema caused by LPS, while the decrease in mean arterial pressure and the increase of hematocrit was partly prevented.

Zymosan is a ligand found on the surface of fungi, like yeast, and it is widely used to activate the alternative pathway of complement. It is a glucan with repeating glucose units connected by β -1,3-glycosidic linkages,

which activates nuclear factor- κ B (NF- κ B) signaling in resident macrophages via Toll-like receptors (15). Damas et al. performed a series of studies to explore the hemodynamic, pulmonary and hematologic effects of zymosan in rats, and reported the following changes (16–21). Intravenous treatment with zymosan reduced serum C hemolytic activity and caused leukopenia, thrombocytopenia, as well as decreased blood pressure and increased hematocrit as a result of extravasation of extracellular fluid in various vascular beds (16–21). Most importantly, zymosan increased right ventricular systolic pressure and respiratory rate (18), which is also a key finding in pigs after liposome administration (22). WEB 2086, a PAF antagonist, prevented the decreases in blood pressure and right ventricular systolic pressure, while indomethacin decreased the tachypnea and pulmonary hypertension but enhanced the drop in blood pressure and right ventricular systolic pressure. The vascular permeability change in the lung was abolished by indomethacin, and no plasma extravasation was found in rats made leukopenic by rabbit anti-neutrophil serum. On the other hand, WEB 2086, the antihistamine, mepyramine or the non-selective serotonin antagonist, methysergide did not affect the vascular permeability response to zymosan in the lung. The zymosan-induced paw edema was prevented by pretreatment with the histamine H2 receptor antagonists, cimetidine and metiamide (19).

CARPA in pregnancy

The potential C activation-related harmful effects of nanomedicines can be even more serious in pregnancy, as sustained C activation is suspected to contribute to the development of gestational complications and preeclampsia (23, 24). Hypertension was induced in pregnant rats using the reduced uterine perfusion pressure (RUPP) model, and the animals were treated daily with the C5a receptor antagonist (C5aRA), PMX51 (acetyl-F-[Orn-P(D-Cha)-WR]), the C3a receptor antagonist (C3aRA), SB290157 (N2-[(2,2-diphenylethoxy) acetyl]-L-arginine) on gestational days 14–18. Both C3aRA and C5aRA partially reversed hypertension on gestational day 19, while only the C5aRA lowered tachycardia and attenuated the impaired endothelium-dependent relaxation in the mesenteric artery (25). However, neither antagonist altered the decrease in plasma VEGF concentration, fetal retardation, but the C5aRA decreased the number of circulating neutrophils.

Characteristics of liposome-induced CARPA in rats

The syndrome called later as CARPA was first demonstrated in conscious rats by Rabinovici et al. (1989), who studied the hemodynamic, hematologic and blood chemistry effects of liposome-encapsulated hemoglobin (LEH), a potential red blood cell substitute (26). Intravenous injection of LEH induced a transient, but relatively long lasting (<120 min) hypertension and tachycardia that was accompanied by increases in hematocrit and white blood cell count, while platelet count decreased. Plasma thromboxane B2 (TXB2, the stable metabolite of TXA2) levels increased in inverse correlation with platelet count. Injection of the hemoglobin-free liposome vehicle caused hypotension and tachycardia, increased hematocrit, white blood cell count and plasma TXB2 levels but decreased platelet count (26, 27). In a subsequent study LEH was prepared using synthetic distearoyl phosphatidylcholine instead of hydrogenated soy lecithin. This change in formulation reduced the effects on heart rate and plasma TXB2 levels, while administration of lyophilized LEH had no detectable hemodynamic, biochemical or hematologic effects (27, 28). These results established that the size and compositions of liposomes are key modifiers of the hemodynamic and hematologic changes. The same authors also showed that pretreatment of rats with BN 50739, a platelet-activating factor (PAF) blocker prevented the LEH-induced CARPA, suggesting that PAF is a key mediator of CARPA in rats (29).

Treatment with LEH and, to a lesser extent, hemoglobin-free liposomes reduced plasma hemolytic C activity within a few minutes that came together with a reciprocal increase of plasma TXB2 levels in rats (30). In an attempt to explore the mechanism of C activation LEH was incubated in rat serum in the presence of EGTA/Mg⁺⁺, which inhibits C activation via the classical pathway, or the serum was preheated to 50°C, which inhibits C activation via the alternative pathway. Since heating alone prevented C consumption by LEH, it was concluded that LEH activated the alternative pathway (30). Furthermore, administration of soluble C receptor type 1 (sCR1), or C depletion using cobra venom (CVF) factor prevented the LEH-induced increase in plasma TXB2 levels. These results established a causal relationship between LEH-induced C activation and the release of TXB2 (31). In a later study it was revealed in conscious rats that treatment with liposome vesicles containing anionic phospholipid-methoxypolyethylene glycol (mPEG) conjugates decreased serum hemolytic C activity and increased plasma TXB2 levels, while the nonionic,

methylated phospholipid-mPEG was free of such effects. Therefore, C activation was due to the zwitterionic phospholipid head-groups that should be avoided in order to produce safer vesicles for site-specific drug delivery (32).

Another research group packed contrast agents in liposomes of various lipid compositions in order to prevent glomerular filtration of the contrast agents, and thereby lengthening their circulation time. As to the high dose of contrast agents to be administered, the amount of liposomes was also high. Not surprisingly 300 mg/kg i.v. hydrogenated soy phosphatidylcholine (HSPC) or 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) induced drastic hypotension, decreased total peripheral resistance (TPR) and cardiac contractility (33). On the other hand, soy phosphatidylcholine (SPC) or the addition of cholesterol to DSPC reduced the hemodynamic effects of liposomes at the same dose. However, C activation parameters were not followed. In a subsequent study liposomes were injected i.v. in either maleic acid/NaOH, or Na₂-EDTA (pH 6.65), or Tris-HCl buffer (pH 7.33) and also contained 300 mg/kg iopromide at a 1 to 1 lipid-to-drug ratio. The acidic preparation induced hypotension, decreased TPR and cardiac contractility, while the buffered preparation was much less effective. Acetylsalicylic acid prevented the hemodynamic effects (34). The authors concluded that the size, electric charge, and composition of liposomes were of major importance to elicit cardiovascular responses (34, 35).

In our laboratory we have applied the rat model of CARPA to investigate the immunological and hemodynamic responses to intravenous (i.v.) bolus injections of liposomes differing in surface properties. Systemic arterial blood pressure (SAP) and heart rate (HR) were continuously recorded in anesthetized male Wistar rats, and blood samples were taken to measure blood cell count and plasma TXB2 levels, as well as to determine total C activation using the classical C hemolytic (CH50) assay. The small unilamellar vesicles used in these studies, i.e., commercial Ambisome and a synthetic saturated PC (DPPC) and cholesterol-containing PEGylated liposome formulation wherein 2K-PEG is conjugated to cholesterol (Chol-PEG), had nearly identical size and polydispersity, but had very different surface properties that represented two frequently applied surface modification. Namely, AmBisome is a surface conjugate-free, highly anionic (negatively charged) liposome, while Chol-PEG liposomes are neutral, surface-stabilized stealth vesicles (Table 1). To induce CARPA, zymosan was utilized for direct C activation, while AmBisome and 2K-PEG-Chol (Chol-PEG), as mentioned above, served as liposomal C activators.

We have measured and compared the hemodynamic and hematologic effects of these liposomes in

Table 1: Characteristics of liposomes applied in the study by Dézsi et al. (11), reproduced with permission.

Name	Character	Lipid composition	Mole ratios	Size, nm	PDI	Zeta potential, mV
AmBisome	Anionic, no PEG	HSPC/Chol/DSPG/Vit-E/Amph-B	49:23:18:0.3:9	98	0.12	-53.5
Chol-PEG	Neutral-PEGylated	DPPC/DSPE/Chol/2k-PEG-Chol	62:5:28:5	97	0.05	0.49

comparison with those caused by zymosan that served as positive control. The effects of zymosan administration at 10 mg/kg i.v. are shown in Figure 1. A gradual decrease in SAP by 40% after 10 min (Figure 1A) could be observed, while HR (Figure 1B) did not change. We have also seen significant leukopenia by 50% at 5 min that was restored by 30 min (Figure 1C), which was associated with significant thrombocytopenia by 30% after 1 min (Figure 1D). There was a severe reduction (by 60%) in hemolytic activity (Figure 1E), while plasma TXB2 exhibited significant, 4-fold rise (Figure 1F).

AmBisome, calculated on its phospholipid (PL) content, was applied to rats at 22 mg PL/kg i.v.

Administration of this lipid vesicle lead to a gradual decrease in SAP by 40% after 5 min (Figure 2A), while no change in HR (Figure 1B) was found. However, significant initial leukopenia by 50% at 5 min, switching to leukocytosis by 10 min (Figure 2C) could be observed. This change paralleled the thrombocytopenia by 60% after 3–5 min (Figure 2D). At this high dose, we have seen a reduction in hemolytic activity by 40% (Figure 2E), however, plasma TXB2 rose only minimally (Figure 2F). Except for somewhat different hematologic (Figure 2C, D) and less TXB2 (Figure 2F) changes, the effect of 22 mgPL/kg AmBisome was essentially identical to that seen with 10 mg/kg zymosan.

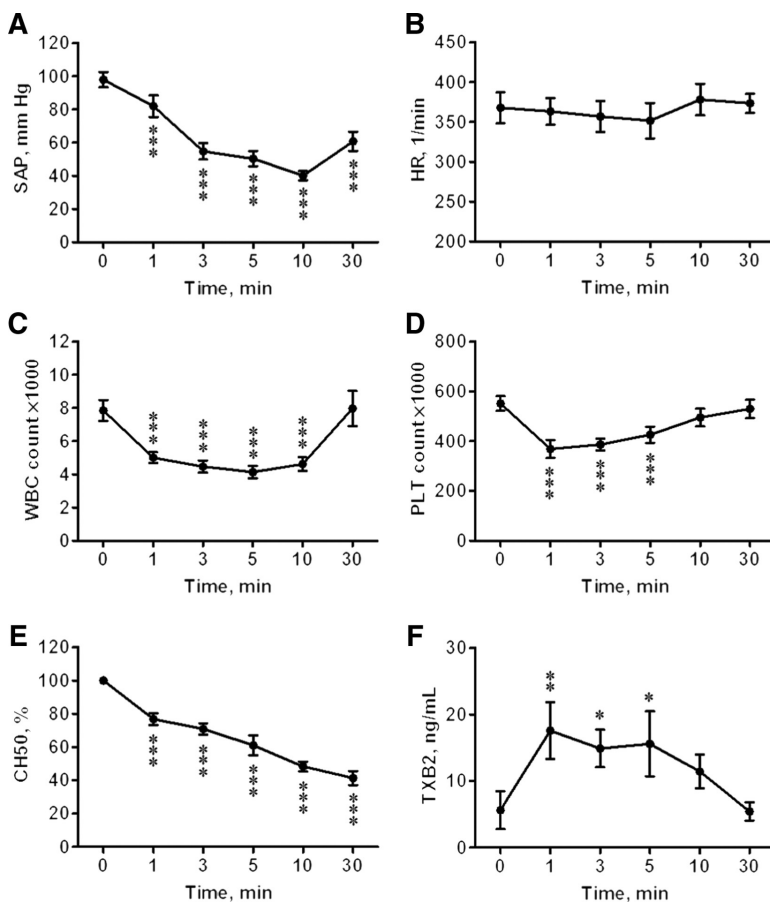


Figure 1: Physiological changes in rats injected with 10 mg/kg i.v. zymosan. Dézsi et al. (11), reproduced with permission. Values shown are Mean±SE (n=8). The curves were constructed from the 0, 1, 3, 5, 10 and 30 min readings of SAP and HR after injection, as well as of other parameters measured from blood samples taken at the same time points. *, **, ***: p<0.05, 0.01, 0.001 vs. the time 0 value.

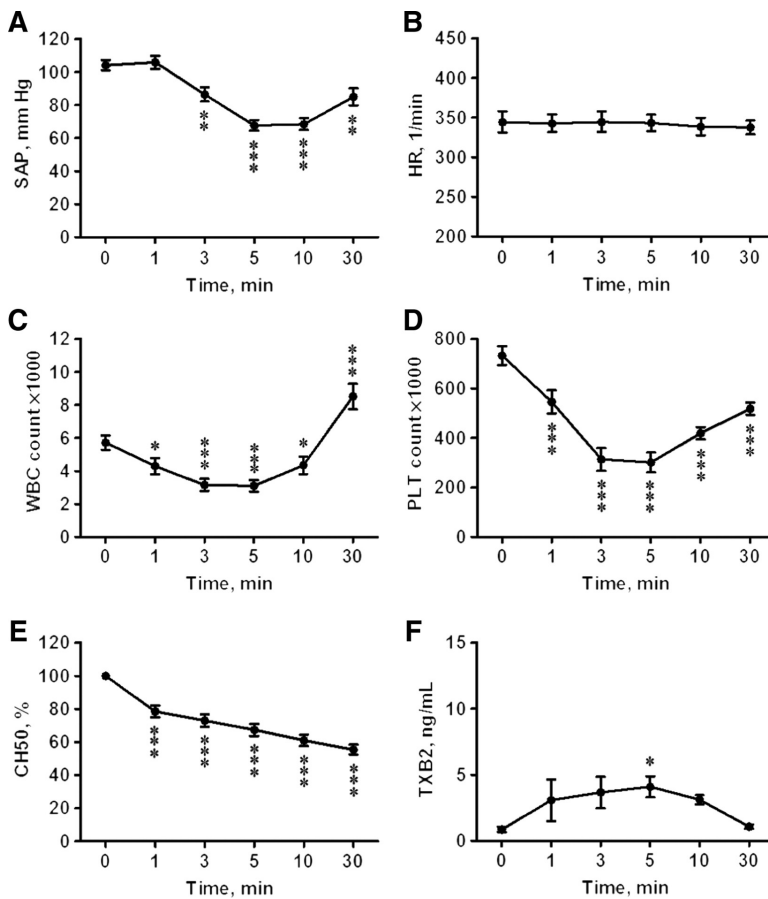


Figure 2: Physiological changes in rats injected with 22 mg PL/kg i.v. AmBisome (n=8). Dézsi et al. (11), reproduced with permission. Similar experiments to that of Figure 1.

Zymosan, being a well-known C activator, the practical identities of the measured physiological effects and C activation by AmBisome and zymosan provides strong support for C activation underlying the observed hemodynamic and hematologic changes.

Finally, the efficacy of the two types of liposomes with different surface characteristics was compared. Chol-PEG liposomes at the dose of 60 mg PL/kg i.v. caused no changes in the measured parameters. Then the effects of a 5-fold higher dose (300 mg PL/kg Chol-PEG) were tested that also resulted only in minor changes. There was relatively small, although significant decrease in SAP (by 16%), while HR did not change. In parallel, we have found moderate leukopenia (but no leukocytosis), as well as thrombocytopenia (both 30%). A slight decrease in hemolytic activity and a small rise in plasma TXB2 could also be observed. Thus, Chol-PEG liposomes turned out to be substantially less effective C activators in rats compared with AmBisome or zymosan.

This study confirmed previous claims that rats are less sensitive to liposome-induced reactions than pigs.

For example, the effective AmBisome dose to induce a similar drop in SAP was 2200-fold higher in rats than in pigs, since 0.01 mgPL/kg i.v. was already effective in pigs. Another notion is that there could be huge differences between test agents of similar kind based on their physical characteristics. These figures provide strong evidence that the rat is not a sensitive model for immune toxicity screening or quantitative evaluation of the risk of CARPA. However, because the physiological changes in rats are essentially the same as those seen in pigs and humans, rats still provide a good model to study the reaction mechanisms of CARPA.

Effects of complement components C3a and C5a in the guinea pig

Complement C3a and C5a have distinct hemodynamic effects. Administration of porcine C5a or C5a des-Arg caused an immediate and short lived fall in blood pressure

followed by a longer hypertensive response lasting for a few minutes in anesthetized guinea-pigs. Only the hypertensive effect was attenuated upon repeated administration, i.e., showed tachyphylaxis (36). Bronchoconstriction followed the same time-course as hypotension, but the reaction was also tachyphylactic (36). The hypertensive effect was similarly, but only partly reduced by pretreatment with histamine or alpha-adrenoceptor blockers, suggesting that C5a caused catecholamine liberation through the release of histamine. The second, hypertensive phase of the C5a effect is specific for the guinea pig as C5a causes mainly hypotension in other species. Administration of C5a des-Arg induced hypotension that lasted for more than 10 min, and was prevented by indomethacin pretreatment. Later studies extended the above findings by demonstrating that indomethacin, the thromboxane synthetase inhibitor U-63557A and the thromboxane receptor antagonist SQ 29,548 all attenuated, but the LTD4 antagonist L-649,923 failed to alter the C5a or C5a des-Arg-induced hypertension in anesthetized guinea-pigs. In summary, the tachyphylactic histamine and catecholamine release and the consequent hypertensive response induced by C5a was mediated by thromboxane. Contrary to the above findings, pyrilamine, a H1 antagonist did not alter, but phentolamine, an alpha-adrenergic antagonist inhibited the hypertensive effect. Treatment with C5a also decreased PMN and platelet counts. The C5a or C5a des-Arg-induced blood pressure rise was diminished after depleting the animals' platelets and white blood cells, while depleting the circulating PMN only had similar effects (37, 38).

The pulmonary response in anesthetized guinea-pigs to intravenous recombinant human C5a (rhC5a) was a reduction in dynamic lung compliance and an increase in pulmonary resistance. Similar to previous studies (39), bronchoconstriction followed the same time-course as hypotension, and it was also tachyphylactic. Bronchoconstriction was not altered by pyrilamine despite an increase in plasma histamine levels. SQ 29,548, a selective thromboxane antagonist decreased the peak response only, while the superoxide dismutase and TXA2 inhibitor U-63557A altered the time course of the bronchoconstrictor response (39). The time course and magnitude of bronchoconstriction was not affected by selective depletion of PMN, platelets or both. However, pyrilamine inhibited the bronchoconstriction after depletion of both circulating PMN and platelets (38). Thus, similar to the hypertensive response, an increased pulmonary resistance due to bronchoconstriction was most likely mediated by the effect of thromboxane, while granulocytes and platelets were less important.

Similarly to C5a, treatment with C3a-peptide (the last 21 amino acids of guinea pig C3a) caused a huge increase in pulmonary resistance and a decrease in dynamic lung compliance in guinea pigs (40). C3a-peptide also induced a transient systemic hypotension, followed by a longer hypertension lasting for about 5 min, and then blood pressure decreased for an additional 10–15 min. All these responses were absent in the C3a receptor-deficient (C3aR-) guinea pigs. Administration of recombinant human C5a (rhC5a) evoked almost the same responses to C3a-peptide with similar magnitude and time course in normal and C3aR- guinea pigs. The ovalbumine-induced increase in pulmonary resistance and decrease in dynamic lung compliance was slightly delayed but was not attenuated in C3aR- vs. normal guinea pigs. On the other hand, the triphasic blood pressure response was altered in C3aR- guinea pigs in such a way that the hypertensive effect was aggravated and delayed and the late hypotensive effect was attenuated, suggesting a minor role of C3aR in the anaphylactic response in guinea pigs (40).

Effects of complement components C3a and C5a in the rat

Treatment with C5a desArg (5 µg/rat) decreased mean arterial pressure and PMN, monocyte and platelet counts but did not alter hematocrit in rats (14). Administration of rhC5a induced an almost immediate hypotension lasting for more than 2 h, and resulted in a shorter, <30 min drop of circulating PMN cell count in rats (41). On the other hand, treatment with rhC3a caused a rapid, dose-dependent hypertension that lasted for a maximum of 5 min. The hypertensive effect was slightly potentiated by pretreatment with carboxypeptidase N inhibitor but was abolished by indomethacin. Administration of rhC3a elevated PMN cell count with a delay of about 90 min at low doses, while at high doses it similarly elevated PMN cell count that was preceded by a small neutropenia lasting for about 60 min, but this early response failed to reach the level of statistical significance. Pretreatment with carboxypeptidase N inhibitor abolished the delayed increase in PMN cell count that was caused by the low dose of rhC3a, but elicited an early and small neutropenia that was similar to that induced by the high dose of rhC3a (41).

One of two short peptide C5a agonist bound to C5aR on both PMN and macrophages while the other had affinity only for the macrophage C5aR. As a consequence, both C5a agonists decreased blood pressure of anesthetized rats, while only the agonist with affinity for the

granulocyte C5aR caused neutropenia (8). These results gave an insight into the mechanism of the various effects of C activation, and raise the possibility of selectively altering various consequences of CARPA.

There are only a few studies on the effects of C in the pulmonary vasculature in rats. Therefore it is an important observation that the infusion of the 21-carboxy-terminal peptide of C3a (C3a57-77) caused an immediate pulmonary vasoconstriction lasting for 10 min in isolated, crystalloid buffer-perfused rat lungs. There was a parallel increase in lung-effluent TXB2 level, which was directly responsible for the pulmonary vasoconstriction as both indomethacin and the thromboxane synthetase inhibitors CGS 13080 and U63,357 inhibited the pulmonary arterial pressor response (42). These results call the attention to the fact that the rat lung can be a direct target organ of CARPA despite the absence of pulmonary intravascular macrophages (PIM cells), which cells seem to have a pivotal role in making the lungs the primary responder organ in the highly CARPA sensitive pigs (11).

Effects of complement components C3a and C5a in the rabbit, hamster and mouse

In anesthetized rabbits treatment with C5a induced a systemic hypotension lasting for about 10 min, a fall in white blood cell count, and an increase in plasma histamine, PGE2, TXB2 and prostacyclin levels, while heart rate, cardiac contractility, hematocrit and platelet count did not change (43). PMN cells almost fully disappeared from the blood, while lymphocyte cell count decreased by about 50%. Central venous pressure increased in parallel with hypotension. All effects remained the same upon repeated administration of C5a. Pretreatment with indomethacin abolished the hemodynamic and prostaglandin responses but leukopenia reappeared. In contrast, the H1-receptor antagonist pyrilamine, and the thromboxane synthetase inhibitor dazoxibene failed to alter the hemodynamic responses, while the H2-receptor antagonist cimetidine attenuated the blood pressure drop and the elevations in plasma prostaglandin levels (43).

A detailed study evaluated leukopenia and subsequent leukocytosis activity of human C5a in a rabbits. Neutrophil, monocyte, eosinophil, and basophil counts all rapidly dropped upon intravenous treatment with human C5a, suggesting the C5a-activated leukocytes which become adherent, leading to sequestration, and depletion

of cells from the circulation. However, C5a seem to mobilize bone marrow causing a huge increase in monocyte, eosinophil, and basophil counts starting at 10–20 min after treatment. Indomethacin failed to alter the effects of C5a. Epinephrine, dexamethasone, lipopolysaccharide, and the prostanoid 15(S)-15-methyl PGF2 α produced a different profile of leukocyte mobilization than that of C5a. It was hypothesized that C5a was directly responsible for the leukocytosis without the involvement of secondary mediators (44).

The hamster cheek pouch is a suitable model for studying the microvascular effects of C3a and C5a. Topical application of C3a (10 nM) caused local vasoconstriction, platelet aggregation, and increased vascular leakage of fluorescein-labeled dextran. Higher doses of C5a (20 or 100 nM) had the same effects also resulted in accumulation of PMN. Pretreatment with mepyramine, a histamine H1 receptor blocker partially inhibited the early phase (up to 5 min) of complement-induced extravasation, which was also partly due to recruitment of PMN (45).

Liposome-induced CARPA was rarely evaluated in mice *in vivo* but the result of a number of excellent *in vitro* tests have been published, among others a study by Banda et al., showed that iron-containing nanoparticles can activate complement in mouse serum via the lectin and alternative pathways (46). A recent *in vivo* study has shown that intravenous treatment with a polyethoxylated castor oil-free, liposome-based paclitaxel formulation or paclitaxel-free liposomes caused hypersensitivity reactions after treatment, including shortness of breath and dyspnea. The paclitaxel formulation induced pulmonary edema, and increased serum sC5b-9 and lung histamine, i.e., caused C activation (47). These results suggest that in mice also the lung seems to be the primary target organ similarly to pigs (22). The role of zymosan causing plasma extravasation was widely investigated in the zymosan-induced peritoneal inflammation model in mice. Zymosan increased vascular permeability and caused peritoneal inflammation in Balb/c mice. The role of mast cells was found to be crucial in the zymosan-induced peritonitis, which was mediated via histamine receptors (48). Other authors have found that leukotriene C4 synthase (LTC4S) mediates an increased vascular permeability (49). These effects were lacking in mast cell-deficient WBB6F1 Balb/c mice and LTC4S^{-/-} C57BL/6 mice (48, 49).

Although we consider the liposome-induced CARPA as a side-effect due to C activation, liposomes can activate PMN leukocytes that can lead to a therapeutic effect. In fact, empty liposomes were similarly effective to liposomal amphotericin B in alleviating invasive pulmonary aspergillosis in a non-neutropenic murine model (50).

Table 2: Symptoms of CARPA in different rodent species.

Symptoms	Rat	Mouse	Guinea pig	Rabbit
Rise of PAP (dyspnea)	+ (18)	+ (47)	+ (36, 39, 51)	+ (52)
Hypo/hypertension	+ (11)	+ (53)	+ (36)	+ (43, 54)
Hemoconcentration (rise of Hct)	+ (14, 26)	+ (53)	?	?
Leukopenia/leukocytosis	+ (11)	+ (55)	+ (38)	+ (43, 54)
Thrombocytopenia	+ (11)	+ (56)	+ (38)	+ (54)
Rise in TXB2	+ (11)	?	?	+ (43, 54)

The presence of specified symptoms are shown with +mark, with corresponding references in brackets. ? means lack of information.

Characteristic changes of in vivo observed parameters in various rodent models of CARPA are summarized in Table 2.

Conclusions

Although the effects of treatment with nanoparticle-containing carrier systems and medicines have not been fully explored in all rodent species, the collective results unequivocally prove that all major characteristics of CARPA are present in rodents including respiratory, hemodynamic and hematologic effects. The current information clearly shows that rodents represent appropriate models to study the reaction mechanisms of CARPA, as well as rodents are suitable for safety prediction of side effects of nanomedicines in humans, although at a much lower level of sensitivity.

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