Journal of Advanced Biomedical and Pharmaceutical Sciences

Journal Homepage: http://jabps.journals.ekb.eg

Complement Activation-Related Pseudo Allergy of PEGylated Products: Safety Aspects, Models, the Role of Anti-PEG Antibodies, and Ways to Overcome

Mahmoud Mostafa[1,3*], Nehal E. Elsadek[3], Sherif E. Emam[2,3], Hamdy Abdelkader[1,4], Usama Farghaly Aly[1], Hatem A. Sarhan[1]

[1] Department of Pharmaceutics, Faculty of Pharmacy, Minia University, Minia 61519, Egypt.
[2] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Zagazig University, Zagazig 44519, Egypt.
[3] Institute of Biomedical Sciences, Tokushima University, 1-78-1 Sho-machi, Tokushima, Japan.
[4] Department of Pharmaceutics, College of Pharmacy, King Khaled University, Abha, Saudi Arabia.

Received: December 29, 2021; revised: January 16, 2022; accepted: January 17, 2022

Abstract

PEGylated products were reported to suffer accelerated blood clearance (ABC) phenomenon upon injection into the same animal twice. The PEG moiety is recognized by the immune system and induces antibodies production. Anti-PEG antibodies can bind PEGylated products and form immune complexes. This interaction activates the complement system and attaches to the preformed immune complex. The larger immune complexes were captured by the liver to be eliminated. Complement activation is considered vital for the ABC phenomenon. As well, complement activation can induce allergic reactions upon injection of several approved pharmaceutical ingredients or drug delivery systems resulting in the so-called "complement activation related pseudo allergy". CARPA accompanied with an injection of the approved PEGylated products is common. In this review, we tried to discover the correlation between the ABC phenomenon and CARPA. Complement activation resembles the meeting point of the two phenomena. Based on our investigations, we can conclude and confirm that the anti-PEG antibodies play a pivotal role in the induction of most CARPA symptoms associated with PEGylated products. Unfortunately, naturally occurring anti-PEG antibodies have a widespread between population which may carry potential CARPA upon consumption of PEGylated products. In conclusion, the expression of CARPA manifestations for PEGylated products is controlled by several factors like the pre-existence of anti-PEG antibodies, anti-PEG antibodies titer, and antigen-antibody ratio

Keywords
PEG, anti-PEG antibodies, ABC phenomenon, complement activation, CARPA

1. Introduction

Hypersensitivity reactions are group of immediate adverse effects mediated by the immune system and might be accompanied with administration of different pharmaceutical products (1-4). The hypersensitivity reactions that might be expressed minutes to hours after systemic, intravenous, injection of the pharmaceutical products is usually called infusion reactions (IRs) (5). The mechanism beyond IRs is not fully understood but it seems that the activation of the complement system plays the predominant role in the initiation and exacerbation of these reactions and so-called complement activation related pseudo allergy (CARPA). CARPA represents a major obstacle against the proper development of several safe intravenously injected pharmaceutical active ingredients (6). Moreover, CARPA resembles an annoying feature of several approved systemic medicaments as well as radiocontrast agents (7-10). Chest pain, breathlessness, flushing, fever, and rash are the most common symptoms for IRs (11). Occasionally, symptoms of IRs are expressed at higher intensity and develop anaphylactoid reactions that might be lethal (12). CARPA were found to be common with several intravenously injected medicaments/drug delivery systems like liposomes, emulsifiers, micelles, contrast media agents, enzymes, monoclonal antibodies, iron supplements, micro and nano capsules (11, 13). Thus, extensive *in vitro* and/or *in vivo* immune-toxicology studies should be accomplished to predict the potential IRs of systemically injected drugs (14).

Polymer drug/drug delivery system modification is considered an emerging technique to improve the pharmacokinetic profile of drugs (15, 16). PEGylation, covalent attachment to polyethylene glycol (PEG), was used to enhance drug circulation and avoid immune drug recognition (17). PEG, a repeated ethylene glycol unit, is considered a non-immunogenic polymer that upon conjugation to proteins, enzymes or drug carriers ensure its prolonged half-lives via diminishing the systemic clearance by reducing macrophage drug uptake, reduce their toxicity, and prevent nonspecific adsorption (18-20). Also, PEGylation helped in masking the potential immunogenicity of the therapeutic proteins or drug carriers while preserving their therapeutic effectiveness (21, 22). However, and in contrary to the general concept that PEG is a non-immunogenic molecule, PEG might elicit immune response, even under certain conditions. Recently, natural "pre-existing" anti-PEG antibodies were detected massively in healthy volunteers who had never challenged PEGylated products (8, 9, 23, 24). Such findings could support and build a body of evidence that PEG could be immunogenic. Detection of pre-existing anti-PEG antibodies showed an

**** Correspondence: Mahmoud Mostafa***
***Tel.**: 01017234040**; Fax:** +2086-236-90-75.*
***Email Address:** mahmoud_mohamed@mu.edu.eg*

increase with time. Anti-PEG antibodies were detected in about 1% at 1984 and reached about 45% at 2016 (23, 25). Such increase in about two decades could be attributed to excessive use of PEG in food, cosmetics and pharmaceutical industry (26) or the increased analytical methods sensitivity (27, 28).

This review discusses with some detail's infusion reactions especially CARPA including the mechanism, manifestations, factors that might control the presentation of CARPA symptoms and CARPA animal models. The safety concerns of CARPA were also discussed to understand and evaluate the potential complement related threats. Also, the tests used to predict CARPA were reviewed to help picking up the proper medicament and/or drug delivery system for each individual case. Accordingly, the underlying factors that might initiate and provoke CARPA, including the role of antidrug antibodies, were reviewed for better management of CARPA cases. Up to our knowledge, this is the first review that discuss the role of antidrug antibodies on the presentation and experience of CARPA symptoms.

Mechanism of infusion reactions

Infusion reactions may occur by single or different overlapping mechanisms. Gell and coombs (29, 30) have classified hypersensitivity reactions into four types according to underlying causes (**Table 1**). One of the best studied mechanisms that outline infusion reactions is CARPA which is considered anaphylactoid reactions in which IgE is not involved while mast cell activation and cytokine release may be prompted (31). Anaphylatoxins (C3a/C5a) released after complement activation provoked by some liposomal formulations e.g., Doxil mediate mast cells and basophils degranulation (13, 32). IV infusion route make the identification of hypersensitivity type impossible at clinical situations but type III could be distinguished by immunohistochemistry and histopathology of immune complexes.

Mechanism of CARPA

The exact mechanism of IRs is not well understood but it appears that complement activation act as the keystone in their onset and so named "complement activation related pseudo allergy" (CARPA) (33, 34). Unlike IgE-mediated anaphylactic reactions, CARPA usually occurs upon the first exposure with rapid short-term manifestations (minutes to hours) and coupled with complement activation (13, 35-37). CARPA symptoms include tachycardia, shortness of breath, wheezing, urticaria, fever, sweating and respiratory distress (38). There are two proposed mechanisms that could explain CARPA, over expression of anaphylatoxins or double hit hypothesis. Regarding anaphylatoxin overexpression, complement activation usually result in massive production of anaphylatoxins (C3a and C5a) which are well-known to have receptors on numerous cells like blood cells and inflammatory mediators secreting cells (39). Activation of these receptors by anaphylatoxins produce activation of platelets, white blood cells, mast cells, macrophages, and basophils (39, 40). Secretions of such cells result in promotion of endothelial cells and smooth muscle cells and finally pulmonary vasoconstriction, systemic vasodilatation, bronchoconstriction, and coronary vasoconstriction (**Figure 1**) (40). This proposed pathway outlines CARPA as trigger "anaphylatoxin" dependent phenomenon. Such proposal is not quite true because there are several animal species that do not express CARPA tachyphylactic symptoms, notably pulmonary symptoms, unless injected with lethal dose of antigen. Even though, the same animal or animal species could express different tachyphylactic symptoms in respond to the same antigen. In other words, CARPA tachyphylaxis should differ in magnitude not in subject itself if it is only anaphylatoxin dependent. Therefore, another factor might control the expression of tachyphylactic symptoms thus the hypothesis "double hit theory" was arisen (**Figure 2**) (35, 41, 42). The double hit hypothesis propose that there are two signals affect the inflammatory mediators secreting cells: one signal is the anaphylatoxin binding and the other one is the binding of the antigen molecule itself (42). PEG molecule anchored on the surface of PEGylated liposomes can bind to several types of receptors on the surface of inflammatory mediators cells like pattern recognition receptors that consequently contribute in cell degranulation and inflammatory mediators release (35).

CARPA manifestations

Animal model has a widespread use in preclinical trials of therapeutic drugs. However, the use of animal models in the determination of immunogenicity of drugs or antigens is very limited because the immunological reactions of animals and human are quite different (43). But the situation of CARPA is fully different because CARPA is complement dependent syndrome which is common in most of animal models and human as well. Typical CARPA manifestations in porcine animal model (**Figure 3**) include (a) hemodynamic changes: pulmonary hypertension and systemic hypo/hypertension, (b) hematological changes: thrombocytopenia, (c) blood chemistry changes: thromboxane (A_2, B_2) and complement cleavage products increase (C3a, C5a, iC3b, C4d, sC5b-9), (d) skin redness and flush (35, 38).

Factors affecting presentation of CARPA symptoms

There are several factors that might potentate the induction of CARPA by PEGylated formulations like morphology, charge, composition, route, and speed of administration.

The shape is a major contributor that can predict the ability of the formulation to induce CARPA reactions upon administration. Doxil®, PEGylated liposomes doxorubicin, is the most prominent example which can activate complement systems extensively. The high reactogenicity of Doxil® was attributed to the elongated oval shape gained after remote loading of doxorubicin forming a crystal like shape inside the liposomal systems (41). The curvature obtained make the reaction of complement proteins on the particles` surface more easier (41). On the other hand, cisplatin payload into Doxil equivalent PEGylated liposomes (SPI-77) did not changed the spherical shape and consequently no increase in complement activation split products or CARPA symptoms expression (41). Doxil® is characterized by provoking CARPA in a group of people, not all people, but the reason is not fully understood.

Liposomal systems contain high molar concentration of cholesterol (> 45 mol%) can extensively activate complement system and initiate CARPA manifestations leading to circulation collapse and sudden death in 50% of pigs used, while lower cholesterol molar concentration liposomes had no pulmonary reactions (44).

Table 1: Classification of hypersensitivity reactions according to Gell and Coombs classification

Hypersensitivity type	Causes and characteristics	Example	Ref
I	IgE mediated immediate anaphylaxis resulted by histamine release from activated mast cells and basophils. Also, production of leukotrienes, prostaglandins, and thromboxane.	Oxaliplatin formulations	(99)
II	Cytotoxic autoimmune antibodies (IgG and IgM to less extent) against subset of cells resulting in antibody dependent mediated cytotoxicity. Moreover, involvement of complement system, natural killer cells, macrophages, and neutrophils.	Autoimmune hemolytic anemia	(78)
III	Immune complex mediated (mainly IgM – antigen) anaphylactoid manifestations accompanied with complement activation. Further help from neutrophils and platelets.	Anti-drug antibodies e.g., infliximab	(100, 101)
IV	Postponed reactions require presentation of antigens by presenting cells followed by successive T-cell activation, with the help of macrophages, and subsequent cytotoxicity.	Contact dermatitis	(102)

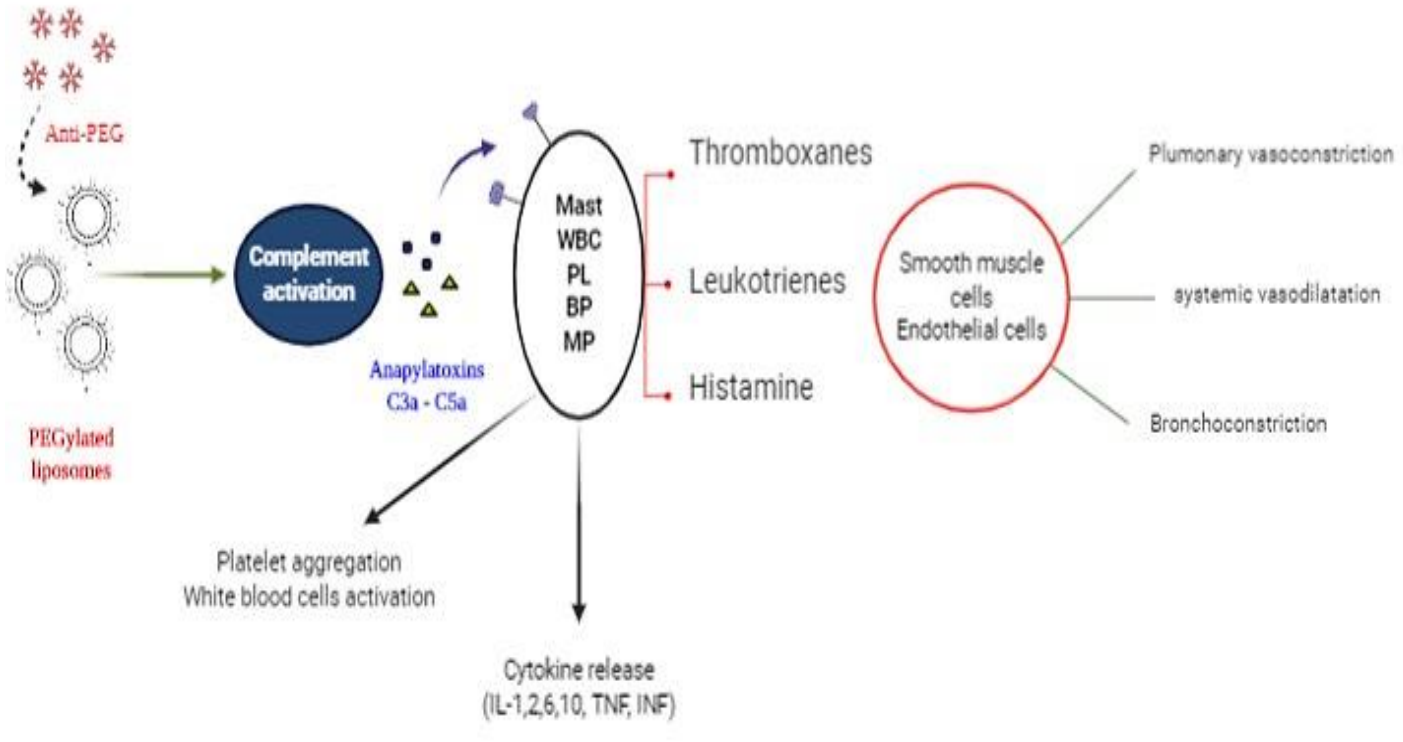

Figure 1: Pathway of CARPA induced by PEGylated liposomes
PEGylated liposomes interact with anti-PEG antibodies followed by complement activation with increase in anaphylatoxins (C3a and C5a) release which have receptors on several immune cells like mast cells, WBCs, platelets, macrophages, and basophils. By anaphylatoxins receptors (ATR) activation these cells begin to release inflammatory mediators like eicosanoids (thromboxane), leukotrienes, histamine, and platelet activating factor. These mediators result in activation of endothelial cells and smooth muscle cell contraction and appearance of pulmonary symptoms. Modified from (55).

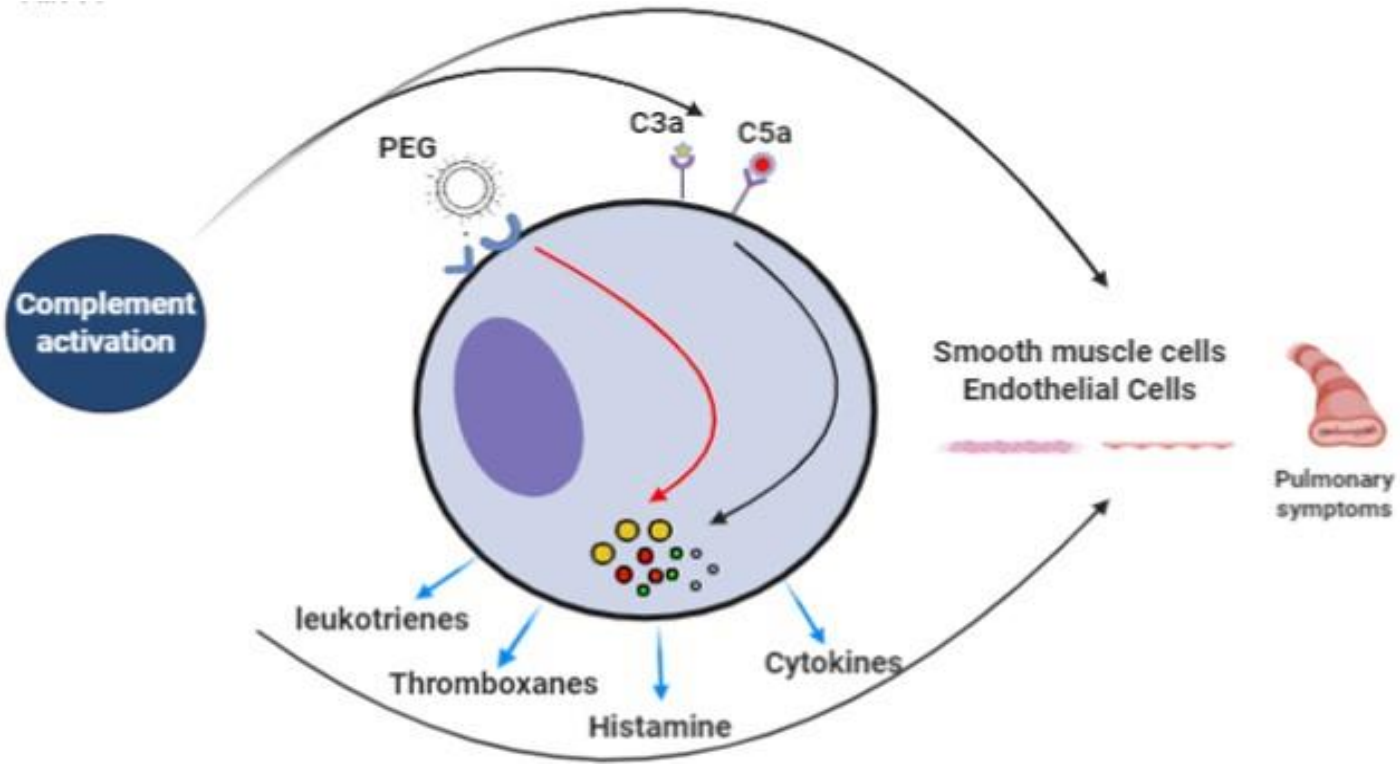

Figure 2: Schematic diagram of double hit hypothesis of CARPA.
CARPA pulmonary symptoms of PEGylated liposomes need two signals to be expressed in pigs. Pulmonary intravascular macrophages (PIM) bind to anaphylatoxins and Doxil through anaphylatoxin and pattern recognition receptors expressed on its surface, respectively. This amplified signal push PIMs toward degranulation and inflammatory mediators' release. Modified from (49).

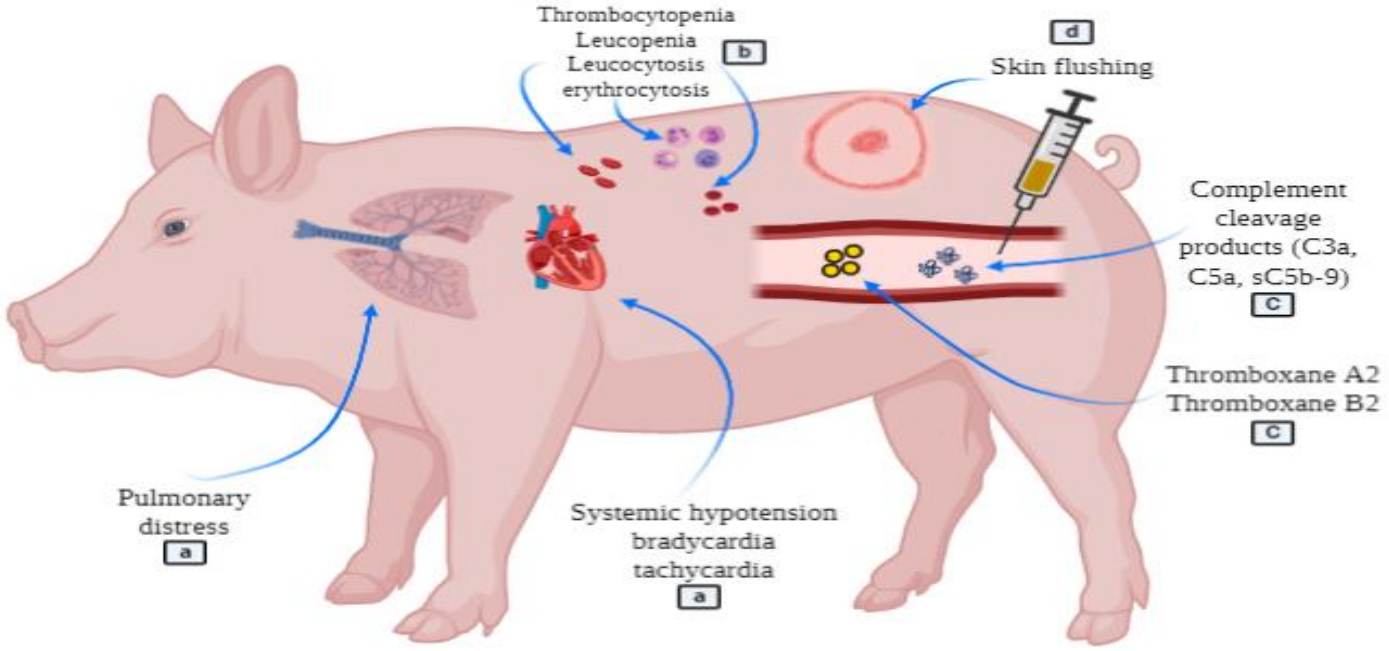

Figure 3: CARPA syndrome in porcine animal model.
Hemodynamic changes: pulmonary hypertension and systemic hypo/hypertension, hematological changes: thrombocytopenia, blood chemistry changes: thromboxane (A_2, B_2) and complement cleavage products increase (C3a, C5a, iC3b, C4d, sC5b-9), (d) skin redness and flush in porcine animal model associated with CARPA syndrome, modified from (35).

The proposed explanation, cholesterol higher concentration form tiny aggregates cannot be accommodated by liposomal system membrane and resemble complement activating points with increased reactogenicity and CARPA expression (44). Also, net charge of liposomal systems plays a crucial role in complement activation. Ambisome®, anionic liposomes used for fungal infections treatment, aggravate intense infusion reactions (CARPA) when compared with equivalent non charged analogues (34). Furthermore, administration of cationic liposomes in rodents resulted in increased levels of complement activation accompanied with hepatotoxicity and pulmonary distress (45, 46).

Complement activation and consequently CARPA symptoms are strictly affected by rate of infusion of nanomedicines. IV bolus injection of non-PEGylated liposomes exerted an increased level of complement activation and pulmonary distress over slow rate IV infusion (44). Since rate of production of anaphylatoxins (C3a and C5a) is dependent upon rate of administration however, rate of clearance is independent on administration method, increased levels of C3a and C5a produced with IV bolus tend to induce exaggerated pulmonary symptoms over slow infusion rate (13, 47).

CARPA animal models

Rodent models (Rats and mice) are usually non sensitive to IRs or CARPA and so need several folds of human dose to express HSRs (34, 48). However, rodents still used in the development and evaluation of several mechanistic studies (49). Successive use of mice, as a model for CARPA, was accomplished by Szebeni et.al for strong complement activators e.g., cobra venom factor, zymosan, and amphotericin B liposomes (49). The study was promising and predictable for human CARPA, especially for the strong complement activators, and could be used to prevent CARPA in man (49). Moreover, rats were successfully used as a model for CARPA, in comparison with pigs, with different complement activators like zymosan, Doxil and Ambisome (34). The dog model is also valuable in screening and estimation of CARPA accompanied with nano-drug infusion at human clinical doses (48). Dogs were efficiently used to assess and evaluate the biocompatibility of drug delivery systems or excipients (50, 51). Nonetheless, CARPA studies need to use large number of animals due to wide inter-variability between individual dogs which may mislead to false positive/negative results (48).

Pig resembles a promising reproducible model for HSRs and CARPA prediction at lower doses equivalent to human one (35). However, the underlaying mechanism/s of HSRs differ from pig to human. Usually, pulmonary intravascular macrophages (PIMs) which are common in pigs play a pivotal role in provoking HSRs, but not in human (52, 53). One assumption for the role of PIMs in human is hepatosplenic migration of macrophages to the lung and exacerbate the common pulmonary symptoms of IRs (54).

Pig and dog models were reported as perfect models for CARPA due to their ideal expression of CARPA manifestations (34, 35, 55). The porcine model showed highly quantitative and sensitive model for CARPA expressing most of CARPA symptoms. A comparison between pig and rat models revealed the superior sensitivity of pig in reproduction of HSRs like human at clinical doses while, rats showed some symptoms at 10-fold dose (34). However, murine models are still used for mechanistic purposes because they are cheaper and accessible (34, 49).

Safety aspects of CARPA

Complement activation not only play a crucial role in initiation of infusion reactions but also represent a safety threat on PEGylated nanomedicines (56). PEGylated liposome doxorubicin suffered untimed release of drug in the presence of anti-PEG antibodies and subsequent complement activation via formation of membrane attack complex between liposomal membrane and sC5b-9 (produced during complement activation) when incubated with human serum from healthy volunteers (57). Preclinical studies using rats confirmed that issue, pre-existence of anti-PEG antibodies activate complement system upon injection of PEGylated liposome doxorubicin and set up membrane attack complex resulting in premature drug release (57). These results build a growing body of evidence, at least in animals, of complement dependent liposomal membrane damage with increased risk of free doxorubicin cardiotoxicity leaked from Doxil® (about 40 % of loaded dose) after complement activation specially with the widespread of natural anti-PEG antibodies (23, 58, 59). Complement mediated membrane damage of Doxil® was poorly reported at clinical situations. There are two possible reasons: first, complement membrane damage affect small fraction of the administered dose with negligible effect on Doxil® pharmacokinetics second, it may arise in high anti-PEG titer sensitive people accompanied with severe infusion reactions and unfortunately poorly reported (56). All these studies confirmed the causal act of anti-PEG antibodies in the development and exaggeration of infusion reactions to PEGylated products (11). Besides, pre-existence of anti-PEG antibodies represent a potential hazard of off target discharge of loaded therapeutics and consequently treatment failure (56).

Prediction of CARPA

There are several tests might be used to predict the CARPA symptoms. Prediction of CARPA and its symptoms might help us to choose suitable therapeutic agents especially for sensitive individuals. These tests include *in vitro* complement activation, basophil, microbalance and biolayer interferometry real time technology tests.

In vitro complement activation: complement cleavage products (C3a, C4d, C5a, iC3b, Bb, and sC5b-9) produced *in vitro* could be used as a CARPA predictor after incubation of normal human serum, plasma, or whole blood with test formulation. However, response of the body to the produced complement split products specially C3a and C5a remains the major contributor in CARPA symptoms expression (55). However, 5-10-fold increase in cleavage product sC5b-9 could be used as a predictor for CARPA, as this fold increase was correlated with CARPA symptoms at several clinical situations (60).

Basophil assay: basophil activation may represent a promising indicator and predictor of CARPA (61-63). Complement activation and subsequent increase in sC5b-9 were correlated with the level of basophil leucocyte activation magnitude (64). Determination of activated basophils was successfully accomplished through detection of CD_{203c} and/or CD_{63} marker/s upregulation using flow cytometry (38, 64).

Microbalance real time technology: recently, quartz crystal microbalance with dissipation was used to support lipid bilayer and complement components were passed over the supported bilayer (65). The attached complement components (MAC) were detected immediately at real time and though could predict the ability of the lipid bilayer (nanomedicine) to activate complement system and provoke CARPA symptoms or not at real time (65). The technology is promising and after optimization might be used

as the major predictor of CARPA expression beside complement activation assay.

Biolayer interferometry real time technology: biosensors were used to bind PEG molecules and immersed in solutions containing complement components in the presence/absence of anti-PEG antibodies (66). The attached complement components increase the capture level and the intensity of the signal recorded at real time. The system could be suitable to predict the CARPA syndrome at short time over ELISA or SDS-PAGE methods.

CARPA: causal role of antidrug antibodies

Several liposomal formulations, either PEGylated or non-PEGylated, had been approved for clinical use and others are under clinical trials at different phases (67, 68). Unfortunately, several liposomal formulations, Doxil®, DaunoXome®, and Ambisome®, activate the complement system upon systemic use and initiate CARPA (41, 60). The reason/s behind these CARPA symptoms is not fully understood since not all patients receiving these liposomal systems got infusion related reactions. The participation of antibodies in triggering CARPA symptoms was studied three decades ago (69). Infusion of cholesterol containing liposomes (43% molar ratio) induced severe HSRs in pigs like cyanosis, respiratory distress, and cardiovascular hemodynamic disturbance within minutes however, cholesterol-free liposomes didn't (69). Such observations supported the involvement of cholesterol autoantibodies in the initiation and aggravation of such infusion/hypersensitivity reactions (69). Besides, injection of PEGylated products e.g. liposomes resulted in consumption of pre-existing or induced anti-PEG antibodies entailing complement activation which was correlated with elevated pulmonary blood pressure (prominent sign of HSRs) (11). These observations support the key role of anti-PEG antibodies, with other minor factors like charge, polydispersity, shape, and size, in initiation and aggravation of HSRs symptoms specially CARPA (8, 9, 11, 13, 41, 70). As well, injection of doxorubicin loaded PEGylated liposomes (Doxil®) in naïve pigs produced light HSRs but on the other hand resulted in life threatening/lethal HSRs in immunized animals during seroconversion period (11). PEG-filgrastim, PEGylated granulocyte colony stimulating factor, was found to induce anti-PEG antibodies upon the first dose in mice followed with accelerated clearance of subsequent doses via complement mediated process (71). Complement activation upon subsequent doses represents potential threatening toward infusion related reactions (71). Regarding clinical studies, pre-existing or induced anti-PEG antibodies may affect the therapeutic activity of the PEGylated products. Pegloticase (PEGylated uricase, refractory gout treatment) therapeutic efficiency was diminished especially upon repeated dose by the induction of anti-PEG immune response (72). Anti-PEG antibodies induced by Pegloticase appeared in about 37% in patients after single injection. PEGnivacogin, anticoagulant PEGylated aptamer that blocks factors IX and Xa, was found to initiate and induce severe infusion reactions within minutes of injections (8, 9). The study confirmed the involvement of anti-PEG antibodies, pre-existed or induced, in the progress of these infusion related reactions (8, 9). Treatment of group of acute lymphoblastic leukemia pediatric patients using PEGylated asparaginase resulted in detection of anti-PEG antibodies, mainly immunoglobulin M (IgM), in about 50% of patients and loss of activity of subsequent doses (25). Moreover, PEGylated asparaginase developed hypersensitivity reactions (HSRs) in about 13% of patients and leaded to drug treatment termination (73). OMONTYS® (PEGinesatide, PEGylated erythropoiesis stimulating factor) was withdrawn two years beyond market release due to severe hypersensitivity reaction within half an hour of infusion with three reported death cases (12). Moreover, Doxil®, PEGylated doxorubicin liposomes, is well-known to provoke infusion related reactions upon the first exposure via a complement mediated process (60). Due to lack of immunological profile of treated patients we cannot exclude the involvement of anti-PEG antibodies in perception of such reactions. Notably, anti-PEG antibodies might be involved in induction of hypersensitivity reactions through antibody-mediated complement activation in pigs, hypersensitivity reaction model for human (11). Besides, several reports focused on the prevalence of anti-PEG antibodies in human beings. In 1984, Richter et. al previewed that about 0.2% of samples had natural anti-PEG antibodies (74). Two decades later, naturally occurring anti-PEG antibodies were detected in about 25 % of normal human doners (75). In one study, about 45% of the subjects showed pre-existing anti-PEG antibodies (23). In another survey study, about 72% of healthy volunteers were positive to anti-PEG antibodies with about 8% exceeding 500 ng/ml barrier (7% IgG and 1% IgM) (59). The reasons for the increased prevalence of naturally occurring anti-PEG antibodies were attributed to excessive exposure to PEG molecules in food, cosmetics, and pharmaceuticals or the improvement of the detection methods over time. Detection of anti-PEG antibodies in plasma samples preserved at blood banks stored from 1970s to 1990s revealed the presence of anti-PEG antibodies in about 56% of sera samples (20% IgG, 19% IgM, and 16% both) (59). That may explain, even in part, the difference in anti-PEG antibodies percent prevalence is due to detection method sensitivity. Collectively, the popularity of anti-PEG antibodies in healthy population could be problematic for proper use of PEGylated products with high opportunity of adverse drug reaction occurrence. Anti-PEG antibodies existence and quantitation surveys before and after treatment using PEGylated medicament are considered beneficial in predicting clinical activity and safety. Therefore, in 2014 FDA recommended monitoring of anti-PEG antibodies in an attempt to diminish clinical efficacy attenuation, adverse consequences, and cross reactivity (76).

CARPA overcoming approaches

To subside nanomedicine induced IRs, we must have a good knowledge about the underlying causes, mechanisms, and effective cells. Such knowledge is not easy to obtain due to complex environment and dual role of some immune cells at different immune reactions. For instance, complement activation play a crucial role in the development of CARPA, type II, and type III hypersensitivity (77-79). As well, cytokines are released at several types of hypersensitivity, CARPA, and cytokine release syndrome (80-82). Such complexity makes understanding of IRs mechanisms very poor and unpredictable. Furthermore, selection of the biomarkers involved in the development of IRs symptoms is not quite easy due to overlapping underlying mechanisms (6, 83). Items should be taken together to get in sight view of nanomedicine IRs (84, 85). First, diagnosis of IRs using clinical manifestations. After that, IgE determination and mast cell markers (histamine and tryptase) were conducted to confirm anaphylaxis. Next, cytokines quantification (interleukin 2, 6, 8, 10, tumor necrosis factor α, and interferon γ) and complement activation split products (C3a, C5a, and sC5b-9) were used to verify non-IgE mediated IRs and CARPA. In addition, naturally occurring antibodies against components commonly used in production of nanoparticles (cholesterol, phospholipids, and

PEG) could generate IRs however, such role needs more studies to be verified (23, 86). A recent group of studies proposed the beneficial use of placebo vesicles (drug free) in avoidance of infusion reactions via minor tachyphylaxis induction (42, 87). Also, drug free vesicles attenuated the ABC phenomenon of subsequent doses of PEGylated products (42, 87). Another group were able to attenuate the ABC effect of anti-PEG antibodies by antibody saturation using high molecular weight free PEG (PEG_{20000}) (88, 89). The diminished ABC was accompanied with minor level of complement activation and though CARPA symptoms (89). Current protocols used to weaken or prevent infusion reactions of PEGylated products e.g., Doxil® include slowing down the infusion rate and use of premeditations (antihistamines and corticosteroids) (56, 90, 91). If all these measures failed to prevent IRs, discontinuation of drug and supportive measurements are required. There are some advanced under research protocols might attenuate or overcome CARPA manifestations (92). These protocols include sensitization using equivalent drug free liposomes, modification of the surface characters of liposomes, use of complement activation inhibitors, and consumption of anti-PEG antibodies.

Sensitization via equivalent drug free liposomes: drug free equivalent liposomes were found to induce tachyphylaxis and prevent infusion related reactions in rodent and porcine models (42, 87).

Modification of the surface characters of liposomes: the surface characters greatly affect the tendency of liposomes to activate the complement system and consequently induce CARPA. The characters include charge and size. Charged and larger liposomes can potentially activate the complement system (93-96).

Complement activation inhibitors: the use of complement activation inhibitor like the naturally occurring factor H which might attenuate complement mediated infusion reactions however it only inhibits the alternative pathway so it might be inefficient at several occasions at which the classical pathway is predominant like antibody mediated complement activation (97).

Consumption of anti-PEG antibodies: the consumption of anti-PEG antibodies might help the attenuation of antibody mediated complement activation (42, 98).

Conclusion

In conclusion, CARPA syndrome might have a direct causal relationship with the pre-existence of antidrug antibodies, natural of induced. This concept might give us a great help and better understanding of the underlying causal factors of these undesirable features accompanied with systemic use of some drugs and /or drug delivery systems. This causal relationship could also explain the paradox of some patients experience CARPA and other patients doesn't upon injection with the same drug or drug delivery system. It seems the experience of CARPA manifestations is related to the antidrug antibody titer and antigen antibody ratio

References

[1] Joerger M. Prevention and handling of acute allergic and infusion reactions in oncology. Ann Oncol. 2012;23:x313-x9.

[2]Johansson SGO, Hourihane JOB, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, et al. A revised nomenclature for allergy: An EAACI position statement from the EAACI nomenclature task force. 2001;56(9):813-24.

[3]Lenz H-J. Management and Preparedness for Infusion and Hypersensitivity Reactions. 2007;12(5):601-9.

[4]Jutel M, Agache I, Bonini S, Burks AW, Calderon M, Canonica W, et al. International consensus on allergy immunotherapy. J Allergy Clin Immunol. 2015;136(3):556-68.

[5]Szebeni J, Simberg D, González-Fernández Á, Barenholz Y, Dobrovolskaia MA. Roadmap and strategy for overcoming infusion reactions to nanomedicines. Nature Nanotechnology. 2018;13(12):1100-8.

[6]Moghimi SM. Nanomedicine safety in preclinical and clinical development: focus on idiosyncratic injection/infusion reactions. Drug Discovery Today. 2018;23(5):1034-42.

[7]Szebeni J, Baranyi L, Savay S, Lutz HU, Jelezarova E, Bunger R, et al. The Role of Complement Activation in Hypersensitivity to Pegylated Liposomal Doxorubicin (Doxil®). Journal of Liposome Research. 2000;10(4):467-81.

[8]Povsic TJ, Lawrence MG, Lincoff AM, Mehran R, Rusconi CP, Zelenkofske SL, et al. Pre-existing anti-PEG antibodies are associated with severe immediate allergic reactions to pegnivacogin, a PEGylated aptamer. J Allergy Clin Immunol. 2016;138(6):1712-5.

[9]Ganson NJ, Povsic TJ, Sullenger BA, Alexander JH, Zelenkofske SL, Sailstad JM, et al. Pre-existing anti–polyethylene glycol antibody linked to first-exposure allergic reactions to pegnivacogin, a PEGylated RNA aptamer. J Allergy Clin Immunol. 2016;137(5):1610-3. e7.

[10]Stewart S, Jablonowski H, Goebel FD, Arasteh K, Spittle M, Rios A, et al. Randomized comparative trial of pegylated liposomal doxorubicin versus bleomycin and vincristine in the treatment of AIDS-related Kaposi's sarcoma. International Pegylated Liposomal Doxorubicin Study Group. 1998;16(2):683-91.

[11]Kozma GT, Mészáros T, Vashegyi I, Fülöp T, Örfi E, Dézsi L, et al. Pseudo-anaphylaxis to Polyethylene Glycol (PEG)-Coated Liposomes: Roles of Anti-PEG IgM and Complement Activation in a Porcine Model of Human Infusion Reactions. ACS Nano. 2019;13(8):9315-24.

[12]DeFrancesco L. Three deaths sink Affymax. Nat Biotechnol. 2013;31(4):270-.

[13]Szebeni J, Muggia F, Gabizon A, Barenholz Y. Activation of complement by therapeutic liposomes and other lipid excipient-based therapeutic products: Prediction and prevention. Advanced Drug Delivery Reviews. 2011;63(12):1020-30.

[14]Hastings KLJIi. Implications of the new FDA/CDER immunotoxicology guidance for drugs. 2002;2(11):1613-8.

[15]Hou Y, Lu H. Protein PEPylation: A New Paradigm of Protein–Polymer Conjugation. Bioconjug Chem. 2019;30(6):1604-16.

[16]Milla P, Dosio F, Cattel L. PEGylation of Proteins and Liposomes: a Powerful and Flexible Strategy to Improve the Drug Delivery. Current Drug Metabolism. 2012;13(1):105-19.

[17]Veronese FM, Pasut G. PEGylation, successful approach to drug delivery. Drug Discovery Today. 2005;10(21):1451-8.

[18]Bazilinski N. Brenner and Rector's The Kidney. JAMA. 1997;277(4):346-.

[19]Veronese FM. Peptide and protein PEGylation: a review of problems and solutions. Biomaterials. 2001;22(5):405-17.

[20]Payne RW, Murphy BM, Manning MC. Product development issues for PEGylated proteins. Pharm Dev Technol. 2011;16(5):423-40.

[21]Abuchowski A, McCoy JR, Palczuk NC, van Es T, Davis FF. Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase. J Biol Chem. 1977;252(11):3582-6.

[22]Abuchowski A, Van Es T, Palczuk N, Davis FJJoBC. Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. 1977;252(11):3578-81.

[23]Chen B-M, Su Y-C, Chang C-J, Burnouf P-A, Chuang K-H, Chen C-H, et al. Measurement of Pre-Existing IgG and IgM Antibodies against Polyethylene Glycol in Healthy Individuals. Anal Chem. 2016;88(21):10661-6.

[24]Hershfield MS, Ganson NJ, Kelly SJ, Scarlett EL, Jaggers DA, Sundy JS. Induced and pre-existing anti-polyethylene glycol antibody in a trial of every 3-week dosing of pegloticase for refractory gout, including in organ transplant recipients. Arthritis Res Ther. 2014;16(2):R63.

[25]Garay RP, El-Gewely R, Armstrong JK, Garratty G, Richette P. Antibodies against polyethylene glycol in healthy subjects and in patients treated with PEG-conjugated agents. Expert Opinion on Drug Delivery. 2012;9(11):1319-23.

[26]Wenande E, Garvey LH. Immediate-type hypersensitivity to polyethylene glycols: a review. Clin Exp Allergy. 2016;46(7):907-22.

[27]Zhang P, Sun F, Hung H-C, Jain P, Leger KJ, Jiang S. Sensitive and Quantitative Detection of Anti-Poly(ethylene glycol) (PEG) Antibodies by Methoxy-PEG-Coated Surface Plasmon Resonance Sensors. Anal Chem. 2017;89(16):8217-22.

[28]Bivi N, Swearingen CA, Shockley TE, Sloan JH, Pottanat TG, Carter QL, et al. Development and validation of a novel immunogenicity assay to detect anti-drug and anti-PEG antibodies simultaneously with high sensitivity. J Immunol Methods. 2020;486:112856.

[29]Pichler WJ. Drug hypersensitivity reactions: classification and relationship to T-cell activation. Drug hypersensitivity: Karger Publishers; 2007. p. 168-89.

[30]Leach MW, Rottman JB, Hock MB, Finco D, Rojko JL, Beyer JC. Immunogenicity/Hypersensitivity of Biologics. Toxicol Pathol. 2013;42(1):293-300.

[31]Luskin AT, Luskin SS. Anaphylaxis and Anaphylactoid Reactions: Diagnosis and Management. Am J Ther. 1996;3(7):515-20.

[32]Szebeni J. Complement activation-related pseudoallergy: A new class of drug-induced acute immune toxicity. Toxicology. 2005;216(2):106-21.

[33]Chanan-Khan A, Szebeni J, Savay S, Liebes L, Rafique N, Alving C, et al. Complement activation following first exposure to pegylated liposomal doxorubicin (Doxil®): possible role in hypersensitivity reactions. 2003;14(9):1430-7.
[34]Dézsi L, Fülöp T, Mészáros T, Szénási G, Urbanics R, Vázsonyi C, et al. Features of complement activation-related pseudoallergy to liposomes with different surface charge and PEGylation: Comparison of the porcine and rat responses. J Control Release. 2014;195:2-10.
[35]Szebeni J, Bedőcs P, Csukás D, Rosivall L, Bünger R, Urbanics R. A porcine model of complement-mediated infusion reactions to drug carrier nanosystems and other medicines. Advanced Drug Delivery Reviews. 2012;64(15):1706-16.
[36]Wibroe PP, Ahmadvand D, Oghabian MA, Yaghmur A, Moghimi SM. An integrated assessment of morphology, size, and complement activation of the PEGylated liposomal doxorubicin products Doxil®, Caelyx®, DOXOrubicin, and SinaDoxosome. J Control Release. 2016;221:1-8.
[37]Lila ASA, Szebeni J, Ishida T. Accelerated blood clearance phenomenon and complement activation-related pseudoallergy: two sides of the same coin. Immune Aspects of Biopharmaceuticals and Nanomedicines: Jenny Stanford Publishing; 2019. p. 771-800.
[38]Szebeni J. Complement activation-related pseudoallergy: A stress reaction in blood triggered by nanomedicines and biologicals. Mol Immunol. 2014;61(2):163-73.
[39]Hugh TE. Structure and function of the anaphylatoxins. Springer Semin Immunopathol. 1984;7(2):193-219.
[40]Mousli M, Hugli TE, Landry Y, Bronner C. A mechanism of action for anaphylatoxin C3a stimulation of mast cells. 1992;148(8):2456-61.
[41]Szebeni J, Bedőcs P, Rozsnyay Z, Weiszhár Z, Urbanics R, Rosivall L, et al. Liposome-induced complement activation and related cardiopulmonary distress in pigs: factors promoting reactogenicity of Doxil and AmBisome. Nanomedicine: Nanotechnology, Biology and Medicine. 2012;8(2):176-84.
[42]Szebeni J, Bedőcs P, Urbanics R, Bünger R, Rosivall L, Tóth M, et al. Prevention of infusion reactions to PEGylated liposomal doxorubicin via tachyphylaxis induction by placebo vesicles: A porcine model. J Control Release. 2012;160(2):382-7.
[43]Brinks V, Jiskoot W, Schellekens H. Immunogenicity of Therapeutic Proteins: The Use of Animal Models. Pharm Res. 2011;28(10):2379.
[44]Szebeni J, Baranyi L, Savay S, Bodo M, Morse DS, Basta M, et al. Liposome-induced pulmonary hypertension: properties and mechanism of a complement-mediated pseudoallergic reaction. 2000;279(3):H1319-H28.
[45]Landen CN, Chavez-Reyes A, Bucana C, Schmandt R, Deavers MT, Lopez-Berestein G, et al. Therapeutic EphA2 Gene Targeting In vivo Using Neutral Liposomal Small Interfering RNA Delivery. 2005;65(15):6910-8.
[46]Ozpolat B, Sood AK, Lopez-Berestein G. Liposomal siRNA nanocarriers for cancer therapy. Advanced Drug Delivery Reviews. 2014;66:110-6.
[47]Lenz H-JJO. Management and preparedness for infusion and hypersensitivity reactions. 2007;12(5).
[48]Szebeni J, Alving CR, Rosivall L, Bünger R, Baranyi L, Bedöcs P, et al. Animal Models of Complement-Mediated Hypersensitivity Reactions to Liposomes and Other Lipid-Based Nanoparticles. Journal of Liposome Research. 2007;17(2):107-17.
[49]Őrfi E, Mészáros T, Hennies M, Fülöp T, Dézsi L, Nardocci A, et al. Acute physiological changes caused by complement activators and amphotericin B-containing liposomes in mice. Int J Nanomedicine. 2019;14:1563-73.
[50]Ribeiro RR, Moura EP, Sampaio WM, Silva SM, Fulgêncio GO, Tafuri WL, et al. Complement activation-related pseudoallergy in dogs following intravenous administration of a liposomal formulation of meglumine antimoniate. 2013;33:1016-20.
[51]Qiu S, Liu Z, Hou L, Li Y, Wang J, Wang H, et al. Complement activation associated with polysorbate 80 in beagle dogs. Int Immunopharmacol. 2013;15(1):144-9.
[52]Schneberger D, Aharonson-Raz K, Singh B. Pulmonary intravascular macrophages and lung health: What are we missing? 2012;302(6):L498-L503.
[53]Winkler GC. Pulmonary intravascular macrophages in domestic animal species: Review of structural and functional properties. 1988;181(3):217-34.
[54]Csukás D, Urbanics R, Wéber G, Rosivall L, Szebeni J. Pulmonary intravascular macrophages: prime suspects as cellular mediators of porcine CARPA %J European Journal of Nanomedicine. 2015;7(1):27-36.
[55]Urbanics R, Bedőcs P, Szebeni J. Lessons learned from the porcine CARPA model: constant and variable responses to different nanomedicines and administration protocols %J European Journal of Nanomedicine. 2015;7(3):219-31.
[56]Gabizon A, Szebeni J. Complement Activation: A Potential Threat on the Safety of Poly(ethylene glycol)-Coated Nanomedicines. ACS Nano. 2020;14(7):7682-8.
[57]Chen E, Chen B-M, Su Y-C, Chang Y-C, Cheng T-L, Barenholz Y, et al. Premature Drug Release from Polyethylene Glycol (PEG)-Coated Liposomal Doxorubicin via Formation of the Membrane Attack Complex. ACS Nano. 2020;14(7):7808-22.
[58]Olson RD, Mushlin PS. Doxorubicin cardiotoxicity: analysis of prevailing hypotheses. The FASEB Journal. 1990;4(13):3076-86.
[59]Yang Q, Jacobs TM, McCallen JD, Moore DT, Huckaby JT, Edelstein JN, et al. Analysis of Pre-existing IgG and IgM Antibodies against Polyethylene Glycol (PEG) in the General Population. Anal Chem. 2016;88(23):11804-12.
[60]Chanan-Khan A, Szebeni J, Savay S, Liebes L, Rafique NM, Alving CR, et al. Complement activation following first exposure to pegylated liposomal doxorubicin (Doxil®): possible role in hypersensitivity reactions. Ann Oncol. 2003;14(9):1430-7.
[61]Ocmant A, Peignois Y, Mulier S, Hanssens L, Michils A, Schandené L. Flow cytometry for basophil activation markers: The measurement of CD203c up-regulation is as reliable as CD63 expression in the diagnosis of cat allergy. J Immunol Methods. 2007;320(1):40-8.
[62]Boumiza R, Debard A-L, Monneret G. The basophil activation test by flow cytometry: recent developments in clinical studies, standardization and emerging perspectives. Clin Mol Allergy. 2005;3(1):9.
[63]Boumiza R, Monneret G, Forissier M-F, Savoye J, Gutowski M-C, Powell WS, et al. Marked improvement of the basophil activation test by detecting CD203c instead of CD63. 2003;33(2):259-65.
[64]Szebeni J. Hemocompatibility testing for nanomedicines and biologicals: predictive assays for complement mediated infusion reactions %J European Journal of Nanomedicine. 2012;4(1):33-53.
[65]Yorulmaz S, Tabaei SR, Kim M, Seo J, Hunziker W, Szebeni J, et al. Membrane attack complex formation on a supported lipid bilayer: initial steps towards a CARPA predictor nanodevice %J European Journal of Nanomedicine. European Journal of Nanomedicine. 2015;7(3):245-55.
[66]Mostafa ME, Nehal E., Emam SE, Ando H, Shimizu T, Abdelkader H, Ishima Y, et al. Using Bio-Layer Interferometry to evaluate anti-PEG antibody-mediated complement activation. Biol Pharm Bull. 2021.
[67]Gabizon A, Goren D, Cohen R, Barenholz Y. Development of liposomal anthracyclines: from basics to clinical applications1This paper is based on a lecture presented at the 8th International Symposium on recent Advances in Drug Delivery Systems (Salt Lake City, UT, USA, 1997).1. J Control Release. 1998;53(1):275-9.
[68]Walsh TJ, Finberg RW, Arndt C, Hiemenz J, Schwartz C, Bodensteiner D, et al. Liposomal Amphotericin B for Empirical Therapy in Patients with Persistent Fever and Neutropenia. 1999;340(10):764-71.
[69]Wassef NM, Johnson SH, Graeber GM, Swartz GM, Schultz CL, Hailey JR, et al. Anaphylactoid reactions mediated by autoantibodies to cholesterol in miniature pigs. 1989;143(9):2990-5.
[70]Szebeni J. Complement Activation-Related Pseudoallergy Caused by Liposomes, Micellar Carriers of Intravenous Drugs, and Radiocontrast Agents. 2001;18(6):40.
[71]Elsadek NE, Lila ASA, Emam SE, Shimizu T, Takata H, Ando H, et al. Pegfilgrastim (PEG-G-CSF) induces anti-PEG IgM in a dose dependent manner and causes the accelerated blood clearance (ABC) phenomenon upon repeated administration in mice. Eur J Pharm Biopharm. 2020;152:56-62.
[72]Sundy JS, Ganson NJ, Kelly SJ, Scarlett EL, Rehrig CD, Huang W, et al. Pharmacokinetics and pharmacodynamics of intravenous PEGylated recombinant mammalian urate oxidase in patients with refractory gout. 2007;56(3):1021-8.
[73]Henriksen LT, Harila-Saari A, Ruud E, Abrahamsson J, Pruunsild K, Vaitkeviciene G, et al. PEG-asparaginase allergy in children with acute lymphoblastic leukemia in the NOPHO ALL2008 protocol. 2015;62(3):427-33.
[74]Richter AW, Åkerblom E. Polyethylene Glycol Reactive Antibodies in Man: Titer Distribution in Allergic Patients Treated with Monomethoxy Polyethylene Glycol Modified Allergens or Placebo, and in Healthy Blood Donors. Int Arch Allergy Immunol. 1984;74(1):36-9.
[75]Armstrong J, Leger R, Wenby R, Meiselman H, Garratty G, Fisher T, editors. Occurrence of an antibody to poly (ethylene glycol) in normal donors. Blood; 2003: AMER SOC HEMATOLOGY 1900 M STREET. NW SUITE 200, WASHINGTON, DC 20036 USA.
[76]Parenky A, Myler H, Amaravadi L, Bechtold-Peters K, Rosenberg A, Kirshner S, et al. New FDA Draft Guidance on Immunogenicity. The AAPS Journal. 2014;16(3):499-503.
[77]Dézsi L, Mészáros T, Őrfi E, Fülöp TG, Hennies M, Rosivall L, et al. Complement Activation-Related Pathophysiological Changes in Anesthetized Rats: Activator-Dependent Variations of Symptoms and Mediators of Pseudoallergy. 2019;24(18):3283.
[78]Vyskocil J, Tucek S, Kiss I, Fedorova L, Nevrlka J, Zdrazilova-Dubska L. Type II hypersensitivity reactions after oxaliplatin rechallenge can be life threatening. Int Immunopharmacol. 2019;74:105728.
[79]Miyabe Y, Miyabe C, Murooka TT, Kim EY, Newton GA, Kim ND, et al. Complement C5a Receptor is the Key Initiator of Neutrophil Adhesion Igniting Immune Complex-induced Arthritis. Science immunology. 2017;2(7):eaaj2195.
[80]Verhoef JJF, de Groot AM, van Moorsel M, Ritsema J, Beztsinna N, Maas C, et al. Iron nanomedicines induce Toll-like receptor activation, cytokine production and complement activation. Biomaterials. 2017;119:68-77.
[81]Maggi E, Vultaggio A, Matucci A. Acute infusion reactions induced by monoclonal antibody therapy. Expert Rev Clin Immunol. 2011;7(1):55-63.

[82]Zhang C, Wu Z, Li J-W, Zhao H, Wang G-Q. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. Int J Antimicrob Agents. 2020;55(5):105954.
[83]Szebeni J. Mechanism of nanoparticle-induced hypersensitivity in pigs: complement or not complement? Drug Discovery Today. 2018;23(3):487-92.
[84]Doessegger L, Banholzer ML. Clinical development methodology for infusion-related reactions with monoclonal antibodies. Clinical & Translational Immunology. 2015;4(7):e39.
[85]Sampson HA, Muñoz-Furlong A, Campbell RL, Adkinson NF, Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: Summary report—Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. J Allergy Clin Immunol. 2006;117(2):391-7.
[86]Szebeni J, Wassef NM, Rudolph AS, Alving CR. Complement activation in human serum by liposome-encapsulated hemoglobin: the role of natural anti-phospholipid antibodies. Biochimica et Biophysica Acta (BBA) - Biomembranes. 1996;1285(2):127-30.
[87]Bavli Y, Winkler I, Chen BM, Roffler S, Cohen R, Szebeni J, et al. Doxebo (doxorubicin-free Doxil-like liposomes) is safe to use as a pre-treatment to prevent infusion reactions to PEGylated nanodrugs. J Control Release. 2019;306:138-48.
[88]McSweeney MD, Shen L, DeWalle AC, Joiner JB, Ciociola EC, Raghuwanshi D, et al. Pre-treatment with high molecular weight free PEG effectively suppresses anti-PEG antibody induction by PEG-liposomes in mice. J Control Release. 2020.
[89]McSweeney MD, Price LSL, Wessler T, Ciociola EC, Herity LB, Piscitelli JA, et al. Overcoming anti-PEG antibody mediated accelerated blood clearance of PEGylated liposomes by pre-infusion with high molecular weight free PEG. J Control Release. 2019;311-312:138-46.
[90]Farr KP, Safwat A. Palmar-Plantar Erythrodysesthesia Associated with Chemotherapy and Its Treatment. Case Rep Oncol. 2011;4(1):229-35.
[91]Strother R, Matei D. Pegylated liposomal doxorubicin in ovarian cancer. Ther Clin Risk Manag. 2009;5(3):639-50.
[92]Mohamed M, Abu Lila AS, Shimizu T, Alaaeldin E, Hussein A, Sarhan HA, et al. PEGylated liposomes: immunological responses. Science and Technology of Advanced Materials. 2019;20(1):710-24.
[93]Löhr JM, Haas SL, Bechstein WO, Bodoky G, Cwiertka K, Fischbach W, et al. Cationic liposomal paclitaxel plus gemcitabine or gemcitabine alone in patients with advanced pancreatic cancer: a randomized controlled phase II trial. Ann Oncol. 2012;23(5):1214-22.
[94]Seidl A, Hainzl O, Richter M, Fischer R, Böhm S, Deutel B, et al. Tungsten-Induced Denaturation and Aggregation of Epoetin Alfa During Primary Packaging as a Cause of Immunogenicity. Pharm Res. 2012;29(6):1454-67.
[95]Vázquez-Rey M, Lang DA. Aggregates in monoclonal antibody manufacturing processes. Biotechnol Bioeng. 2011;108(7):1494-508.
[96]Rosenberg AS. Effects of protein aggregates: An immunologic perspective. The AAPS Journal. 2006;8(3):E501-E7.
[97]Mészáros T, Csincsi ÁI, Uzonyi B, Hebecker M, Fülöp TG, Erdei A, et al. Factor H inhibits complement activation induced by liposomal and micellar drugs and the therapeutic antibody rituximab in vitro. Nanomedicine: Nanotechnology, Biology and Medicine. 2016;12(4):1023-31.
[98]Wang L, Su Y, Wang X, Liang K, Liu M, Tang W, et al. Effects of complement inhibition on the ABC phenomenon in rats. Asian J Pharm Sci. 2017;12(3):250-8.
[99]Makrilia N, Syrigou E, Kaklamanos I, Manolopoulos L, Saif MWJM-bd. Hypersensitivity reactions associated with platinum antineoplastic agents: a systematic review. 2010;2010.
[100]Vultaggio A, Matucci A, Nencini F, Pratesi S, Parronchi P, Rossi O, et al. Anti-infliximab IgE and non-IgE antibodies and induction of infusion-related severe anaphylactic reactions. Allergy. 2010;65(5):657-61.
[101]Steenholdt C, Al-khalaf M, Brynskov J, Bendtzen K, Thomsen O, Ainsworth MA. Clinical Implications of Variations in Anti-infliximab Antibody Levels in Patients with Inflammatory Bowel Disease. Inflamm Bowel Dis. 2012;18(12):2209-17.
[102]Pichler WJ. Delayed Drug Hypersensitivity Reactions. Ann Intern Med. 2003;139(8):683-93.