Mini Review

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Blood cell changes in complement activation-related pseudoallergy

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Abstract: The characteristic physiological changes in complement (C) activation-related pseudoallergy (CARPA) include thrombocytopenia, leukocytosis and leukopenia with or without compensatory leukocytosis. In the background of these phenomena it is known that anaphylatoxins, the triggers of CARPA, can activate white blood cells (WBCs) and platelets, and that this activation can lead to the binding of these cells to each other and also to capillary endothelial cells, entailing microthrombus formation and circulatory blockage mainly in the pulmonary and coronary microcirculation. These changes are key contributors to the hemodynamic alterations in CARPA, and can lead to anaphylactic shock. The goal of this review was to catalogue the blood cell changes in man and different animals undergoing CARPA and focus on some details of the molecular and cellular interactions among anaphylatoxins, other C activation byproducts, platelets, WBCs (mainly monocytes), macrophages and endothelial cells and these cells' secretory products during CARPA. By discussing the inhibitors of different steps of the complex interplay between reaction mediators and cell surface receptors, the review might help in identifying possible novel drugs candidates against CARPA.

Keywords: anaphylatoxins; animal models; hypersensitivity reactions; platelets; pseudoallergy; white blood cells.

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Introduction

Complement activation-related pseudoallergy (CARPA), as the name implies, is a non-Ig-E-mediated (pseudoallergic) hypersensitivity reaction (HSR) that is triggered by C activation, or C activation plays a major contributing role. CARPA is best known in the context of nanotoxicity, since nanomedicines, i.e. particulate drugs and agents in the nano (10^{-9}–10^{-6} m) size range often cause such reactions. As reviewed earlier (1–8), and also discussed in other papers of this issue, the phenomenon represents an immune barrier to the clinical use of many promising nanomedicines. In essence, CARPA may be perceived as a biological stress on blood that arises as a consequence of the similarity of nanomedicines to viruses, between which the immune system cannot make difference (8). The entailing acute inflammatory reaction may have triple harms via 1) causing rapid clearance of the drug and, hence, reducing or eliminating its efficacy; 2) causing an acute illness in the host whose most severe manifestation is anaphylaxis; and 3) leading to immunogenicity, which turns drugs into vaccines and thus abolishes their therapeutic use (9, 10).

The symptoms of CARPA include haematological changes, namely thrombocytopenia, leukocytosis and leukopenia with or without compensatory leukocytosis. This review focuses only on these changes highlighting what is known about their mechanism and what is not. A clear vision in this regard will help formulating the right questions in future research in this field. The particular processes analysed and questions addressed are 1) the effects of anaphylatoxins on blood cells; 2) homo- and heteroaggregate formation between WBC and platelets resulting in microthrombi; and 3) adhesion of the latter to endothelial cells and sequestration in the lung and other organs and 4) the vicious cycle of the above processes leading to circulatory shock. An additional goal of the review is to point to a possible, yet unclarified positive feedback mechanism whereby C activation is exacerbated during CARPA due to activated platelet-induced additional C activation.

Blood cell changes in CARPA: human and animal data

Human studies

The best know HSRs whose pathomechanism is likely to involve CARPA are triggered by anticancer drugs administered in micellar solvents, like Taxol or Taxotere, liposomal drugs, like Doxil and AmBisome and radio-contrast agents, like iodine-containing contrast media. Clinical reports on haematological changes caused by the above drugs often list *thrombocytopenia and leukopenia*, but usually these changes represent cytotoxic, rather than the immune reactogenic effect of the drugs. However in the case of radiocontrast media, early association of *leukopenia* has been attributed to the immune reactivity of the contrast agent (11, 12).

Studies in pigs

Figure 1A and B show the platelet and WBC changes observed in pigs that were treated with bolus injection of large multilamellar liposomes (MLV) (13). The figure shows 10%–30% *drop of platelets and WBC counts* within 30 min after injection in six of eight animals. It is also seen that the individual variation of changes is substantial. Further studies in pigs analysing blood cell changes during long-term infusion of PEGylated small unilamellar liposomes (SUV) showed initial *leukopenia followed by leukocytosis*, i.e. typical roller-coaster pattern of anaphylatoxin-induced WBC changes (14–16).

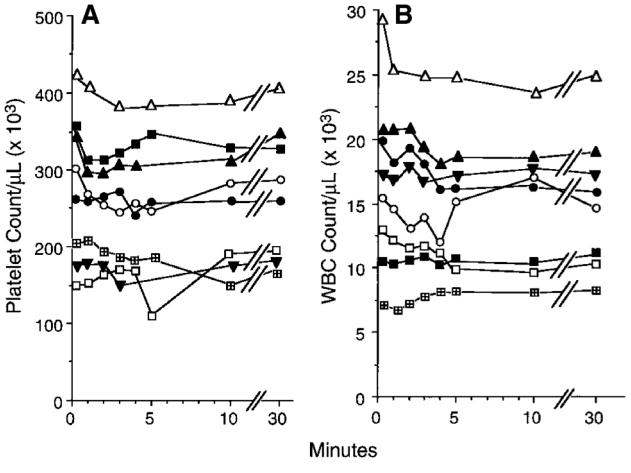

Figure 1: Liposome-induced changes in platelet (A) and white blood cell (WBC, B) counts in pigs. Cell counts were determined before injection of MLV and at different times thereafter, as indicated. Different symbols designate individual pigs. Figure reproduced from (13) with permission.

Studies in rats

Liposomes and other C activators cause CARPA in rats, too, which phenomenon was first described and analyzed in detail by Rabinovici et al. in a series of studies starting in the late 1980s (17–19). They injected liposome-encapsulated hemoglobin (LEH), a red cell substitute, along with appropriate controls, in conscious, normovolemic or exsanguinated rats and studied the hemodynamic and haematological consequences of treatment. LEH, as well as the carrier liposomes, caused *thrombocytopenia*, whose extent depended on the mode of LEH administration, namely, a 10% top load (17), 50% exchange transfusion (18) and 10% top load with lyophilized LEH (19) led to 60, 40 and 24% *thrombocytopenia*, respectively. Also in rats the above thrombocytopenia was associated with a *rise*, rather than drop *of WBC count* (17–19).

Consistent with the above reports 20 years ago, the rat studies in our laboratory using zymosan and liposomal amphotericin B (AmBisome) as reaction inducers, led to 30 and 60% *drop in platelet counts* between 1 and 3 min after i.v. injection of zymosan (Figure 2A) and AmBisome (Figure 2B), respectively. Also, we observed 50% *leukopenia* with zymosan (Figure 2C) and 20% *leukopenia* with AmBisome (Figure 2D), with the WBC counts reaching minimum around 4–5 min. In case of AmBisome, the WBC started to rise over baseline after 10 min (Figure 2D), implying the start of reactive leukocytosis (20). This study confirmed the huge difference between rats and pigs in sensitivity to reactogenic liposomes, as the reactogenic doses in these experiments were 100–1000-fold higher than that reported for pigs (20).

Regarding the destiny of platelets during *thrombocytopenia* in rats, Phillips et al isolated and labeled rat platelets with ^{111}In, then re-infused the cells into the same animal (21). Fifteen minutes later the animals were infused with a 10% top load of LEH, as well as carrier liposomes or free bovine Hb. LEH, but not the controls, caused a transient 50% decrease in ^{111}In platelet activity 2–5 min postinfusion, which returned to baseline levels by 15 min. Tracing of labeled platelets with a gamma scintigraphic camera showed them to be sequestered in the lungs and liver (21).

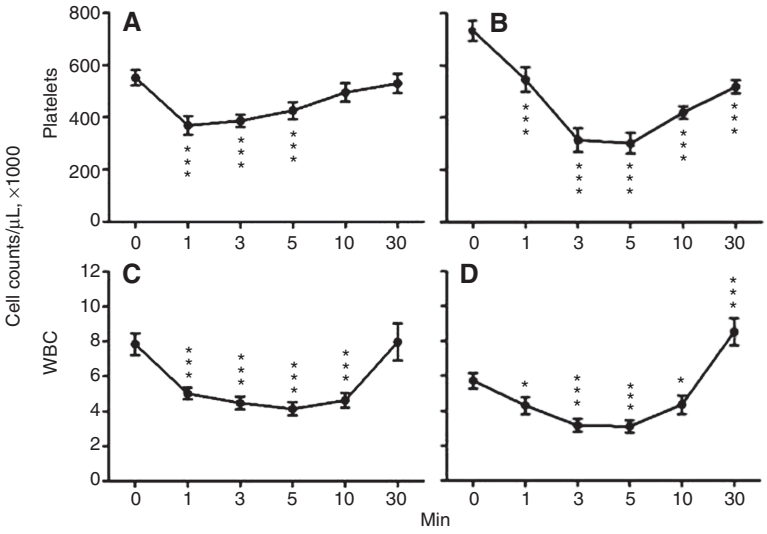

Figure 2: Hematologic effects of iv. bolus administration of zymosan (A, C), and AmBisome (B, D) in rats. Values shown are mean±SE (n=8 rats in each group). *, **, ***: p<0.05, 0.01, 0.001 vs. the time 0 value. The zymosan and AmBisome doses were 10 and 22 mg/kg, normalized to phospholipid amount in case of AmBisome. The figure was modified from Ref. (20) with permission.

The causes and consequences of blood cell changes in CARPA

The role of anaphylatoxins in cell changes

Figures 3 and 4 place the blood cells changes in the complex scheme of CARPA. Figure 3 illustrates that the anaphylatoxins C3a and C5a stimulate mast cells, basophil leukocytes, secretory macrophages, WBC and platelets via their anaphylatoxin receptors to secrete a broad array of vasoactive and inflammatory mediators. These "allergomedins" (2, 3) then act on autonomic effector cells, e.g. smooth muscle cells and endothelial cells causing the circulatory and respiratory changes listed in the rightmost part of the figure.

The fact that that the above listed cells express anaphylatoxin receptors, and that these receptors mediate rapid and massive functional changes have been known and studied for a long time (22–27). Particularly important evidence in this regard is the concentration-dependent, saturable binding of C5a to monocytes/macrophages and neutrophils, leading to myeloperoxidase release, which is an index of degranulation, i.e. release of multiple

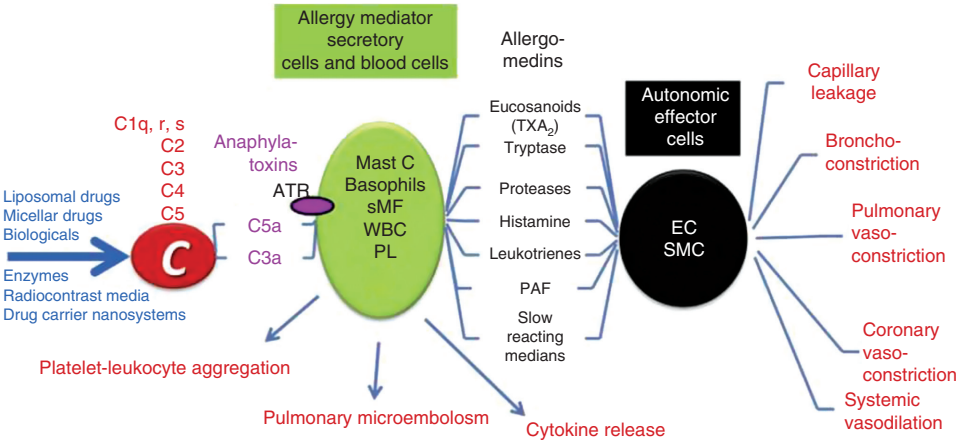

Figure 3: The CARPA cascade. The hypothetical scheme, sketched on the basis of collective information in Refs. (2–4, 22–27), illustrates the steps and interactions among cells and reaction mediators. AR, anaphylatoxin receptors; Mast C, mast cells; sMF, secretory macrophages; WBC, white blood cells; PL, platelets; EC, endothelial cells; SMC, smooth muscle cells. Figure reproduced from (8) with permission.

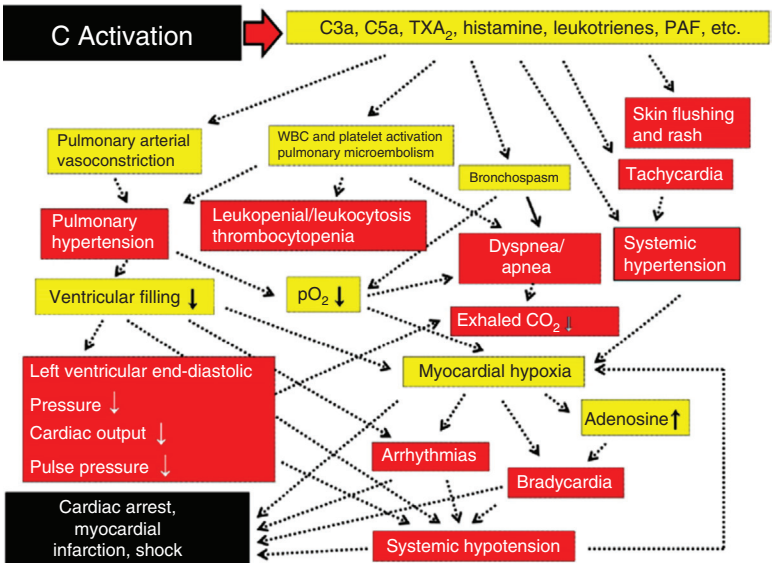

Figure 4: Inter-relations among the different abnormalities during CARPA, leading to clinical symptoms. Hypothetical scheme delineated on the basis of collective information in Refs. (2–4, 22–27). The physiological changes and clinical symptoms are boxed with yellow and red background, respectively. Figure reproduced from (8) with permission.

vasoactive substances (28). Nonmyeloid cells that express anaphylatoxin receptors include endothelial and epithelial cells and smooth muscle cells, responding to anaphylatoxin binding with increased permeability and contraction, respectively (22, 23).

In addition to the above data, there is ample direct experimental evidence for the causal role of anaphylatoxins in CARPA and associated blood cell changes. One such evidence is the inhibitory effect of an anti-porcine C5a antibody (GS1) (29) on liposome-induced porcine CARPA (13), and another one is the prevention of LEH-induced thrombocytopenia in rats by decomplementing the animals (30). The latter experiment showed that in C-depleted rats the platelet counts did not change following injection of LEH, while in control rats 40% thrombocytopenia developed within 4 min after treatment. This was followed by gradual return of platelet counts to normal levels over 60 min. The drop in circulating platelets was correlated with a rapid redistribution of ^{111}In-platelets from the circulation to the lungs and liver, whereas C-depleted rats showed no sequestration of platelets into these organs.

Figure 4 extends Figure 3 inasmuch as it illustrates the complex interactions among blood cells and circulatory and respiratory changes that underlie the clinical symptoms of CARPA. These include, in order of increasing severity: headache, chills, fever, tachycardia, pruritus, rash, flushing, hypotension, hypertension, shortness of breath, apnea, facial swelling, chest pain, back pain, tightness in the chest and throat, arrhythmias, bradyarrhythmia, cyanosis, syncope, anaphylactic shock, myocardial infarction, cardiac arrest, death (2–4). The blood cell changes in the middle of the scheme play an important contributing role to the physiologial changes (22–27).

Platelet changes and interactions during CARPA

Figures 3 and 4 show, among many interactions, that the cascade of cellular and molecular interactions involved in CARPA includes platelet-leukocyte aggregation leading to the formation of leukothrombi, platelet-leuko microemboli, whose pulmonary sequestration can cause circulatory blockade in the lung. These cell transitions and migrations explain their depletion in blood, i.e. the pan-species leuko- and thrombocytopenia discussed above. The next sections provide molecular details of these changes.

Platelets: the mimosa of blood cells

Just like mimosas, platelets are known to be very fragile cells that sensitively and rapidly respond to changes in their milieu. Besides their fundamental role in coagulation, they actively participate in inflammatory reactions and in the pathogenesis of prominent diseases, like

hypertension (31–33), hypercholesterolemia with atherosclerosis (34), thrombosis, asthma (35, 36), complications of diabetes (37, 38) angina pectoris, myocardial infarction and stroke (39). Platelet activation may occur as a consequence of various stimuli, e.g. high shear, physical activity, alterations of the endocrine and homeostatic milieu. Figure 5 shows that the activation of platelets results in shape and surface changes that greatly expand the surface of these cells interacting with each other as well as with endothelium and circulating leukocytes. In addition, stimulated platelets release several soluble mediators, including PF4, PDGF, IL-1β, tissue factor and CD40L.

Platelet-complement interactions

Recent studies have shown that platelets also interact with the C cascade, bridging the coagulation and immune systems. However, importantly, resting platelets do not express anaphylatoxin receptors (28, 40), only activated platelets do it. Along this line, Patzelt et al have shown that the expression of anaphylatoxin-receptors on platelets strongly correlated with their state of activation, measured by the expression of activation markers P-selectin and SDF-1 (40). On the other hand, activated platelets not only express anaphylatoxin receptors, but many other molecules that themselves activate C, and therefore accelerate C action. Thus, activated platelets have an intrinsic capacity to activate C, both the classical and alternative pathways. This has been shown when activated platelets were exposed to normal plasma or serum, and C activation was proportional to the extent of platelet activation and depended on the activator (41–45). Platelets can activate C via different mechanisms, which have been proven for both adherent and circulating platelets in suspension. In these C activations P selectin, receptor for the globular head of C1q (gC1qR/p33) and chondroitin sulfate were shown to play key roles (43). What is known about these platelet surface molecules?

P-selectin is a well-known adhesive protein of the platelet mediating interactions with P-selectin glycoprotein ligand-1 (PSGL-1) on the vascular endothelium and leukocytes. P-selectin is stored in the alpha granules of the platelet and translocated to the surface membrane rapidly upon activation, therefore its expression is a classical marker of platelet activity. P-selectin contains 9 C binding domains, one of which has been identified as a C3b binding site. Ligation of P-selectin leads to C3b deposition, C3a generation, and C5b-9 formation and was shown to be sufficient to activate the C cascade without any further stimuli. Besides P-selectin, alpha granules also contain C1 inhibitor and factor D that cleaves factor B to its active form. Therefore, when P-selectin is presented on the surface of platelets, C activation via the classical pathway becomes restricted and the alternative pathway becomes dominant (43, 46).

The receptor for the globular head of C1q (gC1qR) is expressed on the surface of adherent platelets and platelet microparticles. Collagen binding induces enhanced surface expression of the receptor, while other agonists fail to exhibit such function. The gC1qR molecule contributes to in situ classical pathway C activation (43, 47), but it also has been shown to interact not only with C1q but also with a variety of structurally distinct ligands, such as proteins of the blood coagulation system [high molecular weight (HMW) kininogen, factor XII].

Chondroitin sulfate has been shown to be stored in platelet alpha granula and gets exposed on the surface of platelets rapidly in response to a variety of agonists, including

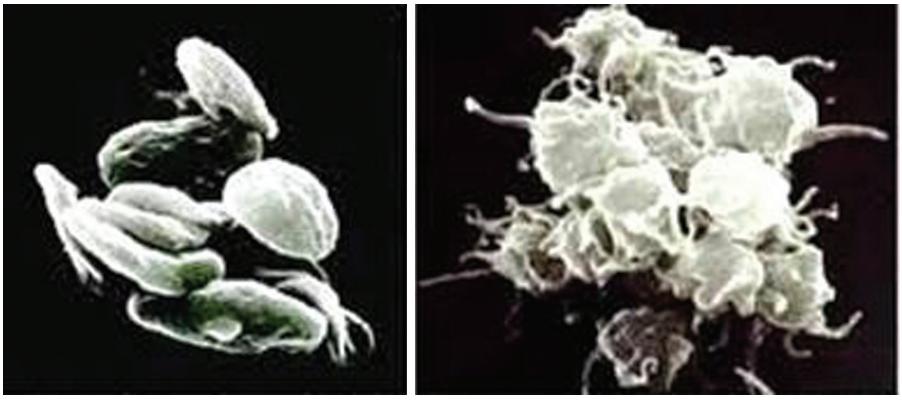

Figure 5: Electron micrographs of resting (A) and activated platelets (B). Figure downloaded from http://vet.uga.edu/ivcvm/courses/VPAT5200/01_circulation/hemostasis/hemostasis03.html.

ADP, collagen, adrenalin and thrombin. As other glucosaminoglycans, chondroitin sulfate facilitates the binding of soluble proteins on the surface. C1q has been shown to bind to chondroitin sulfate in high amounts, while C activation was abolished in the absence of C1q. Following activation with thrombin receptor activating peptide (TRAP), chondroitin sulfate is expressed and C1q, C4, C3 and C9 are bound to the platelet surface (48). As it has been demonstrated recently, C4BP and factor H also bind to platelet chondroitin sulfate-A. The chondroitin sulfate present in the alpha granules of platelets is fully sulfated (48). In a clinical observation, injection of an over-sulfated form of chondroitin sulfate caused fatal anaphlyactoid reaction by activating both the C and the contact systems (49).

Activation of surface adherent, as well as circulating platelets can be triggered by agonists or shear (44). When platelets are activated, C1q, C3b, C4 and C5-9 are deposited on their surface. However, C activation is strictly regulated on platelets, therefore, binding of C elements on the platelet surface does not always result in activation. Platelet activation and consequent C activation may participate in several physiological and pathophysiological processes. Shear induces platelet activation and, in turn, C activation via the classical pathway. Activation of platelets by platelet agonists induces C activation mainly by the alternative pathway. This may result from the mechanism of platelet activation when it results in alpha granule release, which contains C1 inhibitor reducing the classical pathway activation. In this process, weak agonists such as ADP were found to induce less, and thrombin to induce more intense activation of the C cascade.

As a consequence of platelet mediated C activation, activation of the clotting cascade can lead to the generation of C3a and C5a. Beside further activation of platelet functions in a positive feedback cycle, these anaphylatoxins invite leukocytes to the site of platelet activation and activate them (50). This activation may result in the induction of inflammatory pathways as well as formation of heteroaggregates which further enhances the functions of leukocytes and platelets. In the studies of Yin et al. (43), platelet mediated C activation was associated with generation of physiologically relevant levels of inflammatory peptides, C4a, C3a and C5a which support recruitment of leukocytes and increase vascular permeability (46).

Platelet activation by complement

Complement elements interact with circulating platelets, resulting in platelet activation and enhanced coagulation, linking thereby inflammatory and thrombotic events (46). Some elements of the C cascade were shown to be able to activate resting platelets or interact with other platelet-challenging factors. Ligation of C1q receptor leads to platelet activation and elevated surface P-selectin expression. Sublytic concentration of the terminal complex (C5b-9) results in platelet activation by inducing transient membrane depolarisation, granule secretion and generation of platelet microparticles (51–53) as well as translocation of phosphatidylserine to the outer membrane leaflet, enhancing thereby platelet procoagulant activity (53, 54). C3 and C5b-9 have been shown to potentiate thrombin-induced platelet secretion and aggregation. The potent anaphylatoxin, C3a also induces platelet activation and aggregation (55). Platelet microparticles (PMP) can express 50- to 100-fold higher procoagulant activity compared to activated platelets (56). They present glycoproteins Ib, IIb/IIIa, and P-selectin on the surface membrane ready to interact with leukocytes and the vascular endothelium, enhancing thereby vascular inflammation (57–59). Platelet microparticles support in situ C activation via the classical and alternative pathways. Similarly to platelets, PMP express C regulatory proteins on their surface, e.g. C1 inhibitor (C1-INH), as well as CD55 and CD59 (43). C3b and C5b-9 deposition was also observed on PMP (43). Certain bacteria have been shown to be able to activate platelets by a C and antibody dependent mechanism (60) where C proteins and fibrinogen binding of Gp IIb/IIIa were needed for platelet activation.

Leukocyte-platelet aggregation

Leukocytes and platelets are known to interact dynamically in a variety of physiological and pathophysiological processes. Monocyte-platelet aggregates are very sensitive markers of platelet activation; leukocyte-platelet heteroaggregation occurs in a variety of diseases from mild homeostatic changes to acute life-threatening events. Elevated heteroaggregate levels were found in a series of abnormal conditions resulting platelet activation, e.g. high shear, endocrine disorders, diabetes, atherosclerosis, hypertension etc. Platelet activation, however, also results in C activation, as discussed earlier. In spite of the similar mechanism and pathophysiology of these functions, the link between C activation and leukocyte-platelet aggregation has not been intensely studied.

Activated platelets are prone to form heteroaggregates with leukocytes, mainly monocytes. Therefore, elevation of heteroaggregates can be measured in a variety of diseases when platelets become activated. Moreover, formation of platelet-monocyte aggregates has been shown

to be a more sensitive marker of platelet activation than platelet surface P-selectin.

Platelets attach to monocytes in a two-step process, which is mediated by selectins and integrins. As a first step, P-selectin is connected to the leukocyte PSGL-1, which connection is then stabilized by the ligation of the leukocyte integrin Mac-1. On the platelet, the membrane receptor called GPIba (GP-Ib) has been shown to interact with Mac-1. Platelet integrin GpIIb/IIIa also contributes to the stabilization of the complex by Mac-1, when fibrinogen or other ligands, e.g. CD40L are present. This is very similar to the formation of platelet-platelet aggregates when GpIIb/IIIa receptors are connected via fibrinogen. These adhesive interactions result directly in the activation of the participating cells by signal transduction pathways, as it is shown for Mac-1, and, secondly, it allows an enhanced paracrine communication between the cells. Platelets and leukocytes release a series of soluble inflammatory and thrombogenic mediators following the ligation of the participating receptors. As shown in Figure 6, platelet-monocyte heteroaggregation enhances cytokine and prostanoid production by monocytes, as well as their adhesiveness to the vascular endothelium. Moreover, attachment of platelets to monocytes induces CD16 upregulation on CD14+CD16– monocytes resulting in a fenotype change and a higher pro-inflammatory activity.

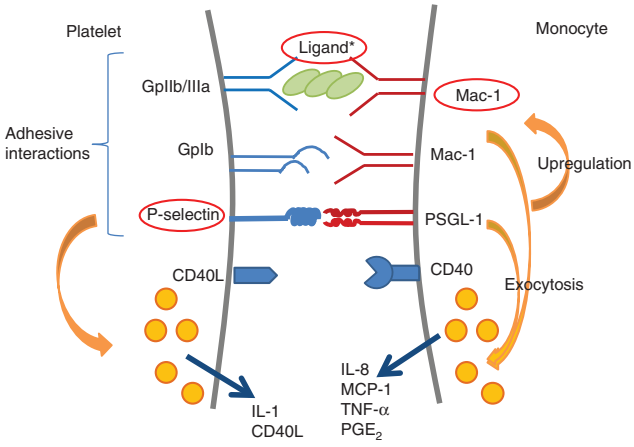

Figure 6: Platelet-monocyte adhesive interactions and inflammatory reactions following complement triggered activation. Platelet-monocyte heteroaggregation is initiated by the ligation of P-selectin to PSGL-1 on monocytes. As a result integrin Mac-1 gets upregulated, and inflammatory mediators are released. Monocyte-platelet complex is then stabilized by Mac-1 which interacts with GpIb and GpIIbIIIa on the platelet. Stabilization via GpIIb/IIIA and Mac-1 needs a soluble ligand present in the plasma or originating from the activated platelet such as fibrinogen or CD40L. CD40L also further increases inflammatory reactions of the monocyte.

Complement activation and leukocyte-platelet interactions

As delineated above, the result of leukocyte-platelet binding is subsequent activation of the participating cells, release of soluble mediators, elevation and activation of surface adhesion molecules and acceleration of thrombotic and inflammatory processes. The soluble mediators originating from activated platelets and leukocytes, as well as the shape change of adherent platelets may contribute to C activation.

Platelets and leukocytes communicate intensively via C activation

P-selectin alone was able to activate the C system, which may be due to its C binding ability. C3b deposition has been observed on platelets upon activation and P-selectin has been identified as a C3b receptor. P-selectin also mediated platelet adhesion to immobilized C3b under flow. This represents a possibility of interaction between the leukocytes (via Mac-1) and the platelet, however, so far P-selectin has been considered to play role in the firm adhesion and Mac-1 in the stabilization of the complex. Moreover, ligation of C3b on platelet P-selectin results in enhanced complement activation and C3a generation with consequent leukocyte activation. C3a also induces platelet activation and aggregation. In addition, sublitic concentrations of C5b-9 result in platelet activation, membrane depolarisation, granule secretion, formation of procoagulant microparticles and phosphatidylserine translocation in the membrane (61). Similarly, an activator function has been assigned to C5b-9 when endothelial cells were exposed to C. These cells defend themselves by exocytosing the attacked membrane portion. Taken together, C activation and its byproducts provide non-contact crosstalk between leukocytes, platelets and endothelial cells via several routes and mechanisms.

Mechanism of pulmonary hypertension in CARPA

Figure 7 illustrates the steps that lead to pulmonary hypertension, at least in pigs (7), based on the discussed multiple interactions among anaphylatoxins, PIM (pulmonary intravascular macrophage) cells, WBC, platelets, and endothelial cells. The anaphylatoxins formed during C activation (only C5a shown) activate the PIM cells, monocytes and PMN, activated platelets and endothelial cells,

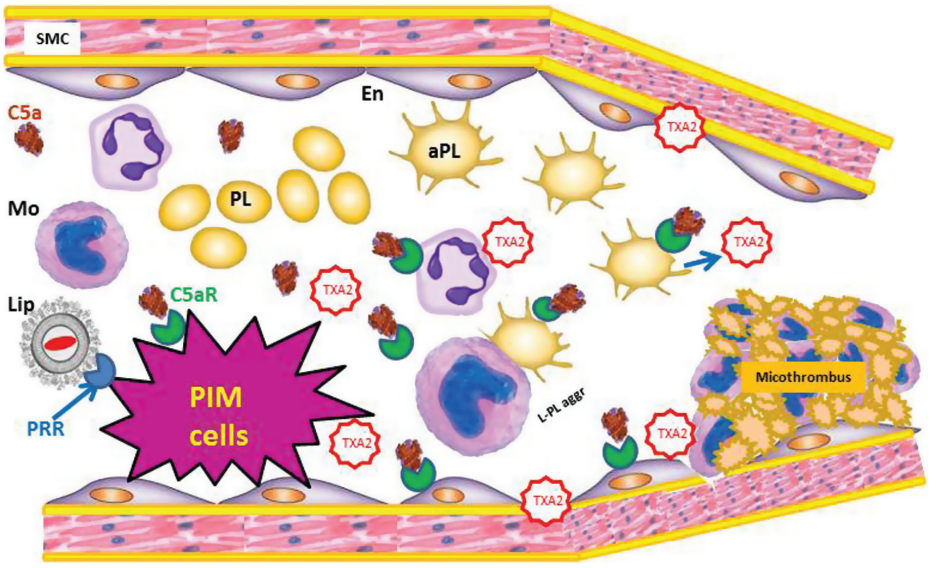

Figure 7: Molecular and cellular interactions underlying the pulmonary hypertension observed in CARPA. The steps are described in the text. SMC, smooth muscle cells; Mon, monocytes; PL, platelets; aPL, Activated platelets; C5aR, C5a receptor; L-PL aggr, leukocyte-platelet aggregates, PRR, patter recognition receptors, Lip, liposomes (Doxil).

resulting the formation of leukocyte-platelet aggregates, which then nucleate microtrombus formation. Anaphylatoxin activation of endothelial cells leads to increased capillary permeability, allowing direct access of smooth muscle cells (SMC) to TXA2, a highly potent vasoconstrictor via causing SMC contraction. Microthrombus formation, together with vasoconstriction, lead to circulatory blockage, and, hence, pulmonary hypertension. Importantly, PIM cells are also activated by direct binding of C activating nanoparticles, e.g. of liposomes to these cells via their pattern recognition receptors, providing a second or "double hit" on these cells to stimulate their degranulation and secretion TXA2 (7).

In addition to CARPA-associated pulmonary hypertension, clinical studies have shown that pathologic conditions with excessive C activation (depleted negative regulators e.g. PNH or HUS) are characterised by thrombosis and platelet hyper-reactivity.

Platelet microparticles

Platelet microparticles (PMP) are released to the circulation following platelet activation. Microparticles exhibit similar activities, and even exceed those exhibited by platelets. PMP can express 50- to 100 fold higher procoagulant activity compared to activated platelets (56). Complement activation occurs in the presence of microparticles and is mediated by the same mechanisms as found for platelets (43). Similarly to platelets, platelet microparticles express gC1qR, C1 inhibitor, CD55 and CD59 on their surfaces. In situ C activation may also contribute to the clearance of activated platelets and platelet microparticles from the circulation, via deposition of C1q and generation of cell surface associated C3b (62).

Relationship between platelet and complement activation disease

It was demonstrated that in patients with elevated cardiovascular risk or enhanced atherosclerosis C elements (C5a) are elevated (63). As shown earlier, in these cases platelets are more activated and elevated monocyte-platelet aggregates (MPA) were measured. As activated platelets not only alter the C functions but at the same time they interact with leukocytes, it would be interesting to study the effect of C elements on leukocyte-platelet aggregate formation, and in turn, the alterations of cell functions following C mediated activation.

Complement and monocyte-platelet aggregates

Despite data suggesting parallel functions of platelet-leukocyte interactions and C activation, the role of

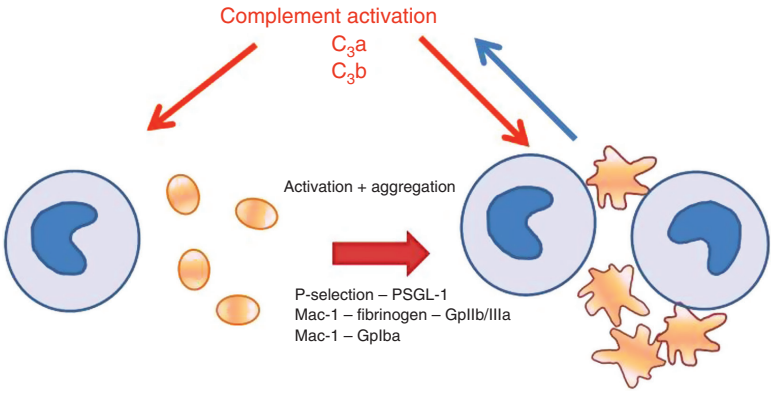

Figure 8: Scheme of interactions between leukocytes, platelets and C proteins.

leukocyte-platelet aggregate formation in anaphylactoid reactions is not clear. Namely, pulmonary hypertension is one of the life-threatening side effects of excessive C activation. In anaphylactoid reactions, obstruction of pulmonary vessels by immune precipitates and aggregates of PMN leukocytes and platelets was observed. It also has been stated that (the second, protracted phase of) anaphylactoid reactions are less intense in leukopenic animals (64). The hypotension observed during anaphylaxis was also more marked in normal than in leukopenic animals.

The administration of C1q inhibitor not only inhibited C, but also leukocyte and platelet activation in a pig to human transplantation model (65). C1q INH also binds to E-and P-selectins and interferes with leukocyte adhesion. Leukocytes and platelets are activated also when pig kidney is perfused with human blood. In this experiment C system was activated and significant leukocyte and platelet activation was observed. Administration of C1-INH/C1 inhibition significantly reduced leukocyte and platelet aggregation (65).

Figure 8 shows that C elements activate platelets and leukocytes which is followed by the interaction of these cells. Activated platelets attach to leukocytes, and this interaction enhances prothorombotic and proinflammatory processes. Soluble mediators derived from this interaction, such as fibrinogen and CD40L stabilize heteroaggregates by ligation of the fibrinogen receptors of both cell types while leukocyte and platelet activation amplify C activation. These processes also lead to the liberation of proinflammatory and prothrombotic mediators and expression of adhesion molecules: P-selectin, GpIIb/IIIa, Mac-1-heteroaggregate formation.

Activation of the C system has been associated with prelesional stages of atherosclerosis and the progression of the disease. Complement elements are deposited in atherosclerotic lesions (66). In endothel C5b-9 promotes the secretion of vWF and elevated expression of P-selectin, E-selectin, intracellular adhesion molecules (ICAM), TF, MCP-1, IL-b, and IL-8 promoting leukocyte adhesion. Complement is also activated in ischaemia-reperfusion injury (67).

Leukocyte and platelet counts in anaphylaxis

In anaphylaxis leukocyte-platelet aggregates are rapidly cleared from circulation primarily because their entrapment in the pulmonary circulation (64). Consequently, interruption of leukocyte-platelet interactions may have important therapeutic consequences to prevent complications of anaphylactoid reactions. One of these possibilities is the administration of GpIIb/IIIa inhibitor abciximab as it not only inhibits the fibrinogen receptor of platelets but also affects Mac-1, thereby (possibly) preventing the stabilization of leukocyte-platelet aggregates.

Summary and outlook

CARPA has traditionally been prevented or inhibited by nonspecific interventions, such as stopping or slowing the infusion, and/or administration of steroids, antihistamines and anti-inflammatory drugs (1-3). By highlighting the various molecular and cellular interactions involved in the process (each of which has specific inhibitors, some are already in the clinics), this review opens opportunity for considering and testing new and specific pharmacological inhibitors of CARPA.

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