Review

Complement activation-related pseudoallergy: A new class of drug-induced acute immune toxicity

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Abstract

A major goal in modern pharmacotechnology is to increase the therapeutic index of drugs by using nanoparticulate vehicle systems in order to ensure slow release or targeted delivery of drugs. With all great benefits, however, these innovative therapies can carry a risk for acute immune toxicity manifested in hypersensitivity reactions (HSRs) that do not involve IgE but arises as a consequence of activation of the complement (C) system. These anaphylactoid reactions can be distinguished within the Type I category of HSRs as “C activation-related pseudoallergy” (CARPA). Drugs and agents causing CARPA include radiocontrast media (RCM), liposomal drugs (Doxil, Ambisome and DaunoXome) and micellar solvents containing amphiphilic lipids (e.g. Cremophor EL, the vehicle of Taxol). These agents activate C through both the classical and the alternative pathways, giving rise to C3a and C5a anaphylatoxins that trigger mast cells and basophils for secretory response that underlies HSRs. Pigs provide a useful model for liposome-induced CARPA as minute amounts of reactogenic liposomes cause C activation with consequent dramatic cardiovascular and laboratory abnormalities that mimic some of the human symptoms. Consistent with the causal role of C activation in liposome-induced HSRs, a recent clinical study demonstrated correlation between the formation of C terminal complex (SC5b-9) in blood and the presence of HSRs in patients treated with liposomal doxorubicin (Doxil). Overall, the CARPA concept may help in the prediction, prevention and treatment of the acute immune toxicity of numerous state-of-the-art drugs.

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Keywords: Allergy; Anaphylactoxins; Anaphylactoid reaction; Micelles; Radiocontrast agents; Cancer chemotherapy; Taxol; Cremophor EL

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Abbreviations: C, complement; CARPA, complement activation-related pseudoallergy; CI-INH, C1-esterase inhibitor; CREL, Cremophor EL; HSR, hypersensitivity reaction; MLV, large multilamellar vesicles; PEG, polyethylene glycol; RCM, radiocontrast media; SC5b-9, S protein-bound C terminal complex

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1. Introduction

Hypersensitivity reactions (HSRs) have been traditionally categorized in four groups from I to IV. Coombs and Gell, authors of this concept defined Type I reactions as IgE-mediated acute reactions, while the rest of categories included subacute or chronic immune changes triggered or mediated by IgG, immune complexes or lymphocytes (Coombs and Gell, 1968). However, it has increasingly been recognized that a substantial portion of acute allergic reactions, whose symptoms fit in Coombs and Gell’s Type I category, are actually not initiated or mediated by pre-existing IgE antibodies. Recent estimates suggest that these non-IgE-mediated “anaphylactoid, pseudoallergic or idiosyncratic” reactions may represent as high as 77% of all immune-mediated immediate HSRs (Demoly et al., 1999), implying hundreds of thousands of reactions and numerous fatalities every year (Szebeni, 2001).

Known examples of pseudoallergy include the reactions caused by radiocontrast media (RCM), non-steroidal anti-inflammatory drugs, analgetics, morphine and insect venoms, liposomes and micellar solvents, such as Cremophor EL (CrEL) in Taxol. While there is no known common underlying cause for most of these reactions, there is substantial evidence suggesting that the reactions caused by RCM, liposomes and CrEL have a common trigger mechanism: complement (C) activation. Thus, HSRs where the allergen can activate C have been tentatively named C activation-related pseudoallergy (CARPA) (Szebeni et al., 1999, 2000a,b). The phenomenon is increasingly recognized as an immune toxicity issue that has particular significance in the modern field of pharmaceutical nanotechnology; R&D of

Table 1

<table>
<thead>
<tr>
<th>IgE-mediated Type I allergy</th>
<th>CARPA</th>
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<tr>
<td><strong>Common symptoms</strong>&lt;br&gt;Angioedema, asthma attack, bronchospasm, chest pain, chill, choking, confusion, conjunctivitis, coughing, cyanosis, death, dermatitis, diaphoresis, dyspnea, edema, erythema, feeling of imminent death, fever, flush, headache, hypertension, hypotension, hypoxemia, low back pain, lumbago, metabolic acidosis, nausea, pruritus, rash, rhinitis, shock, skin eruptions, sneezing, tachycardia, tingling sensations, urticaria, wheezing</td>
<td><strong>Unique symptoms</strong>&lt;br&gt;Reaction arises after repeated exposure to the allergen&lt;br&gt;Reaction is stronger upon repeated exposures&lt;br&gt;Reaction does not cease without treatment&lt;br&gt;Reaction rate is low (&lt;2%)&lt;br&gt;Spontaneous resolution&lt;br&gt;High reaction rate (up to 45%), average 7%, severe 2%</td>
</tr>
</tbody>
</table>
particulate drug carriers, synthetic nano and microcapsules, liposomes and lipid complexes, micellar carriers and emulsifiers, new formulations of radiopharmaceuticals and contrast agents, etc. (Hunter and Moghimi, 2003; Ten Tije et al., 2003; Storm and Woodle, 2003; Barratt, 2003). This increased awareness of CARPA is also reflected by the fact that testing for C activation in vitro and/or in vivo has become one of the immuno-toxicology tests recommended by the US Food and Drug Administration (FDA) that may be useful to identify the pseudoallergy potential of drugs, when needed (Hastings, 2002).

2. Symptoms of CARPA

As listed in Table 1, many symptoms of CARPA are the same as seen in common allergy or classical type I reactions, while others are unique to C activation. Perhaps the most important distinguishing feature of CARPA is the lack of presensitization and reinforcement, i.e., the reaction arises at the first exposure to the drug and then it decreases, rather than increases upon repeated exposure.

3. Complement activation-related pseudoallergy caused by radiocontrast media

3.1. Prevalence and critical factors

Hypersensitivity reactions to RCM, also referred to as “RCM reactions”, have been a concern ever since the first organic, iodinated compound was used for i.v. pyelography in 1928 (Grainger, 2001). Although today, with the use of new-generation RCM, the frequency of severe reactions fell to very low values (see below), the wide use of RCM (in the USA more than 10 million tests are performed yearly (Kumar and Mahalingam, 2001; Hong et al., 2002) still implies a significant number of reactions and occasional fatalities. According to a recent estimate applied for all kinds of symptoms with all procedures and all types of RCM, the overall incidence rate of RCM reactions is 2.1–12.7% (Hong et al., 2002). The frequency of relatively mild cutaneous, vasomotor, pulmonary, cardiovascular or gastrointestinal symptoms is in the 5–8% range (Kumar and Mahalingam, 2001), while life-threatening reactions has been estimated to occur in 0.0004–0.002% of patients, at least in the case of coronary angiography (Kumar and Mahalingam, 2001).

Several factors have been identified to contribute to or influence these reactions, including the osmolarity, charge and association of the molecules in RCM, the speed of its i.v. administration and the health, recent medication and constitutional features of patients. In general, low-osmolarity, nonionic, dimeric or trimeric RCM slowly administered to healthy, nonallergic people carries no, or much less risk for HSR than ionic, high-osmolarity, monomeric RCM administered as a bolus to people who are recovering from an infection and/or prone for allergy (Hong et al., 2002; Westhoff-Bleck et al., 1990; Barrett et al., 1991; Katayama et al., 2001; Henry et al., 1991).

3.2. Pathomechanism of RCM reactions

The pathogenesis of RCM reactions is considered multifaceted, as beside C activation, several other mechanisms and controlling factors were shown to play more or less roles. As illustrated in Fig. 1, mast cells and basophils are in the centre of RCM reactions. They can be triggered by RCM molecules directly, through poorly understood intracellular interactions and/or extracellular physical effects (for example, osmotic stress), or indirectly, via cell membrane receptors. The latter include the FcεR and the anaphylatoxin receptors (C5aR and C3aR), binding IgE, C5a and C3a, respectively. Positive feedback can be provided by co-activation of the coagulation and kinin-kallikrein systems, leading to crossover activation of the C cascade with depletion of C1INH.

Fig. 1. Schematic representation of the multiple factors and effects involved in RCM reactions. Taken from (Szebeni, 2004a,b) with permission of the publishers.
vomiting, hypotension and hyperreflexia. The authors
the injection of sodium iothalamate, manifested in
reported severe "idiosyncratic" response of a dog to
role of C activation in RCM reactions Lasser et al.
As for the effector arm of RCM reactions, secondary mediators include histamine, tryptase, PAF, LTB4, LTB6, LTC4, LTD4, LTE4, TXA2, PDG2 and TXD2 (Westhoff-Bleck et al., 1990; Greenberger, 1984; Lieberman, 1991) (Fig 1). Some of these mediators (e.g., PAF, histamine, tryptase and TXA2) are preformed and liberate from the cells immediately upon activation, while others are de novo synthesized and, hence, liberate slower. Further variations of RCM reactions are due to individual differ-
ences in the expression of cellular receptor subtypes
on which mast cell secretion products act. For exam-
ple, activation of H1 receptors leads to vasoconstriction
and vascular leakage, and is responsible for the cardio-
vascular and cutaneous symptoms of anaphylaxis. H2
receptors, on the other hand, increase cellular cAMP lev-
el and cause vasodilation, increased heart rate and pulse
pressure (Lieberman, 1989).
Another important factor in the secretory response of
mast cells and basophils to RCM is the location of
these cells. For example, mast cells from the skin
may not respond to certain RCM, while pulmonary and
cardiac mast cells are triggered for strong release reac-
tion (Genovese et al., 1994). Likewise, mannitol, via
osmotic stimulus, may induce the release of histamine
from human basophils, but to a lesser extent from mast
cells (Genovese et al., 1994).
3.3. Complement activation as underlying cause of
RCM reactions
RCMs have a complex impact on the C system mainly
via physical effects (charge, viscosity, iodine number, hydrophobicity and osmotic pressure) (Lieberman, 1991; Napolovlu et al., 1998; Vik et al., 1995). Complement activation by RCM was demonstrated to proceed both through the classical and the alternative pathways (Lieberman, 1991; Napolovlu et al., 1998), as well as via uncommon mechanisms, such as non-localized, non-
sequential cleavage of C proteins (Kolb et al., 1978), suppres-
sion of Factors H and I (Lieberman, 1991) and
direct action on the thioester bonds of C4 and C3 (Vik
et al., 1995).
Among the animal studies attesting to a causal role of C activation in RCM reactions Lasser et al. reported severe "idiosyncratic" response of a dog to
the injection of sodium iothalamate, manifested in
vomiting, hypotension and hyperreflexia. The authors
found significant depletion of C during the symptoms,
suggesting that C activations was causally involved
in the reaction (Lasser et al., 1976). In further dog
studies by Lang et al. serial daily injections of RCM
(metrizamide, iothalamate, diatrizoate, acetrizoate,
iodipamide and iopanoate) caused substantial declines
of serum C over several days (Lang et al., 1976). In rats, Napolovlu et al. proved that various RCM in the
0.5–2.0 g iodine/kg range activated the C system via the
alternative pathway, with efficacy in the following order:
triombrast > hexabrics > ultravist > melitrat > omnipac
(Napolovlu, 1997; Napolovlu et al., 1998).
In humans, case reports implicating C activation in
more or less severe RCM reactions gave account of
decreased plasma hemolytic C, C3, C4 (Lasser et al.,
1980), decreased factor B and C1 esterase inhibitor
(CHNH) levels (Lasser et al., 1980; Vandenplas et al.,
1990), rises of C3 conversion products (Lasser et al.,
1980; Vandenplas et al., 1990) and the presence of con-
sumption coagulopathy (Lasser et al., 1980; Vandenplas
et al., 1990), pulmonary capillary leakage (Vandenplas
et al., 1990) and acute respiratory distress syndrome with
granulocytic aggregates in the pulmonary microcircula-
Among the more extensive clinical studies looking
at the role of C activation in RCM reactions, Small
et al. (1982) analyzed HSRs and C activation in 220
patients undergoing i.v. pyelography. Nineteen percent
of patients displayed HSRs, while depressed serum CH50
levels, indicating C activation, occurred in 49%. The
RCM-induced decline of CH50/mL was apparent within
90 s after starting the infusion and returned to normal
after about 30 min. This study highlighted an important
fact regarding the relationship between C activation and
HSRs, namely, that more people display signs of C acti-
vation than HSRs. Hence, C activation may be present
in patients without clinically manifest reaction, suggest-
ing that anaphylatoxin liberation does not necessarily
cause HSRs. C activation may therefore be a precon-
dition, or contributing factor to HSRs, but it does not
solely explain the phenomenon. Other factors or pre-
conditions may also need to be present in people who
develop HSR. This point was reinforced in the studies by
Westaby et al. (1985), who demonstrated significant ele-
vation of the anaphylatoxin C3a in the peripheral blood
of 7/11 patients receiving RCM for coronary angiogra-
phy. In 3/7 patients, C3a was increased between 4- and
10-fold, yet only one of these patients developed symp-
toms, which were mild.
It should be noted that C activation has not been
a consistent finding in all clinical studies reporting
C measurements in patients injected RCM. Kolb et
tions (Szebeni, 2004a,b). Out of the marketed liposomal drugs are in advanced clinical trials, or already used in patients mainly for anticancer and antifungal applications. At present, more than a dozen liposomally delivered drugs are in advanced clinical trials, or already used in patients mainly for anticancer and antifungal applications (Szebeni, 2004a,b). Out of the marketed liposomal drugs Doxil (Caelyx) (Uzuny et al., 1995; Alberts and Garcia, 1997; Dezube, 1996; Gahizon and Martin, 1997; Gahizon and Muggia, 1998; Chanan-Khan et al., 2003), AmBisome (Levine et al., 1991; Lang et al., 1994; Ringdén et al., 1994; de Marie, 1996; Schneider et al., 1998), Abelcet (de Marie, 1996), Amphocil (de Marie, 1996) and DaunoXome (Cabrales et al., 1998; Eckardt et al., 1994; Fossa et al., 1998; Gill et al., 1995, 1996; Girard et al., 1996; Guaglianone et al., 1994; Money-Kyrle et al., 1993; Richardson et al., 1997) have been reported to cause HSRs with symptoms corresponding to CARPA (Table 1). The frequency of HSRs to liposomal drugs shows large variation between 3 and 45% (Szebeni, 1998, 2001).

4.1. Evidence for a role of C activation in liposome reactions

4.1.1. In vitro studies

Since its discovery in the late sixties (Haxby et al., 1968; Alving et al., 1969), C activation by liposomes has been analysed in a great number of studies. The emerging picture is very complex, as variations in liposome structure and other experimental conditions can result in fundamental differences in the extent, pathway and kinetics of activation (Szebeni, 2001, 1998).

Focusing on C activation by Doxil, as an example, its incubation with 10 different normal human sera led to significant rises in C terminal complex (SC5b-9) levels over PBS control in seven sera, with rises exceeding 100–200% (relative to PBS control) in four subjects (Szebeni et al., 2000b). Further experiments showed that in addition to the quantitative variation in SC5b-9 response, Doxil-induced C activation also varied in different individuals in terms of sensitivity to inhibition by 10 mM EGTA/2.5 mM Mg2+, which distinguishes classical from alternative pathway activation (Szebeni et al., 2000b). The minimum effective C-activating concentration of Doxil was 0.05–0.10 mg/mL and there was linear dose–response relationship up to about 0.5 mg/mL. The activation curve reached plateau at doses ≥0.6 mg/mL, suggesting saturation of response (Szebeni et al., 2000b). Doxil also caused variable liberation of Bb, a specific marker of alternative pathway activation, providing further evidence for a role of alternative pathway activation and/or amplification (Szebeni et al., 2000a,b).

These and other studies from our laboratories (Szebeni et al., 1994, 1996, 1997a,b, 1999, 2000a,b, 2002; Szebeni and Alving, 1999) highlighted some basic conditions and mechanism of liposomal C activation. Thus, large size, polydispersity, positive or negative surface charge and high (>45%) cholesterol content were all shown to promote, whereas small uniform size and neutrality reduced the proneness of liposomes for C activation. The process may involve both the classical and alternative pathways, with the latter acting either as the only activation mechanism, or as a positive feedback mechanism amplifying C activation via the classical pathway. As for the classical pathway, the presence of liposome-reactive immunoglobulins represents a powerful trigger or enhancer, but their presence is not a precondition for C activation via this pathway. Direct binding of Clq to the phospholipid bilayer, or to C reactive protein-tagged liposomes, can also activate C via the classical pathway. Thus, C activation by liposomes can involve numerous redundant triggering and controlling processes whose differential manifestations in individuals may explain, at least in part, the substantial variation in vivo responses to liposomes, as discussed below.

4.1.2. Clinical evidence for C activation as underlying mechanism of liposome-induced HSRs

The likely clinical relevance of C activation by liposomes can be deduced from clinical studies in the past, taken together with a recent study dedicated to address the cause–effect relationship between in vivo C activation and clinical reactions to Doxil.

In reviewing the historic evidence, one of the earliest clinical studies with liposomal drugs reported that intravenous infusion of vesicles containing NSC 251635, a water-insoluble cytostatic agent, led to increased C3deficiency in the plasma of cancer patients (Coune et al., 1983). Another, also still indirect proof, is the finding of Skubitz et al. (Skubitz and Skubitz, 1998) on transient neutropenia with signs of leukocyte activation in patients who displayed HSRs to Doxil. As is known, neutropenia with leukocyte activation are classical hallmarks of ana-
phylatoxin action (Cheung et al., 1994; Skroeder et al., 1994a,b).

To the authors’ knowledge the first direct evidence for the causal relationship between C activation and HSRs to liposomes was provided by Brouwers et al. (2000), who reported 3 severe HSRs out of 9 patients obtaining 99mTc-labeled pegylated liposomes for scintigraphic detection of bowel inflammation (Dams et al., 2000). In one reactor patient plasma C3, C4 and factor B decreased by 16–19%, implying major C consumption. The fact that both C4 and factor B were depleted suggests that C activation involved both the classical and the alternative pathways.

The study dedicated to correlate C activation with HSRs gave account of 45% reaction rate in cancer patients infused with Doxil for the first time (Chanan-Khan et al., 2003). The grade 2 or 3 HRSs in 13 of 29 patients occurred in men and women in approximately equal proportions and were not related to the age of patients. Importantly, Doxil caused C activation in 21 out of 29 patients (72%) as reflected by significant elevations of plasma SC5b-9 levels following infusion of the drug. The time course of SC5b-9 increase in blood showed substantial individual variation (Fig. 2), including rapid elevations within 10 min with gradual return to near baseline within 2 h (A), rapid elevation without return within 2 h (B) and moderately rapid elevation of SC5b-9 until about 30 min, followed by partial return to baseline during 2 h (C). The lack of SC5b-9 response is demonstrated in Fig. 2D.

Taking the baseline and 10 min post-infusion SC5b-9 values in clinical reactors and non-reactors, the study reported significant increase of SC5b-9 in 12/13 reactor patients in contrast to 9/16 in the non-reactor group (Fig. 3). Thus, 92% of clinical reactors were also laboratory reactors, while only 56% of clinical non-rectors were laboratory reactors. These data led to the conclusion that C activation and HSR show significant \( P < 0.05 \) correlation.

The quantitative relationship between SC5b-9 values at 10 min and severity of HSR revealed that the SC5b-9 assay is highly sensitive in predicting HSRs (Table 2), although the specificity and positive predictive value of the test was relatively low, particularly in patients in whom the rise of SC5b-9 at 10 min remained below two-times the upper limit of normal SC5b-9 (Table 2, raw 2). However, restricting the criteria for laboratory reactivity to 10 min SC5b-9 values exceed-

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**Fig. 2.** Time course of Doxil induced changes in plasma SC5b-9 in cancer patients and its individual variation. Panels A–D demonstrate data from four subjects displaying different patterns of response. Data are mean ± S.D. for triplicate determinations. (*) Significantly different from baseline, \( P < 0.05 \). Reproduced from (Chanan-Khan et al., 2003) with permission.
Fig. 3. Plasma SC5b-9 levels at baseline and at 10 min post-infusion of Doxil in cancer patients displaying (A) or not displaying (B) HSRs to Doxil. Data are mean ± S.D. for triplicate or duplicate determinations. The dashed lines indicate the normal range of SC5b-9, i.e., the normal mean ± 2 S.D. (*) Significantly different from baseline (P < 0.05). The numbers under the bars are the patient ID. Reproduced from (Chanan-Khan et al., 2003) with permission.

ing two- or four-fold the upper threshold of normal (Table 2, raw 3), the specificity and positive predictive value of the C assay remarkably increased with relatively less decrease in sensitivity. Thus, the extent of SC5b-9 elevation was proportional with the specificity and positive predictive value of the C assay with regards to HSRs.

Finally, the study revealed significant correlation between dose rate and SC5b-9 (P < 0.01), indicating that C activation at 10 min was Doxil dose dependent.

In summary, these data strongly suggested that C activation might be causal or a key contributing, but not rate-limiting factor in liposome-induced CARPA. This proposal in keeping with the mentioned report by Small et al. (1982) wherein 19% of patients infused with a RCM displayed HSR, although C activation was detectable in 49%. A plausible hypothesis explaining the phenomenon is that reactors differ from nonreactors in at least two criteria: (1) they are susceptible for C activation by the drug and (2) their mast cells and basophils have a lower than normal threshold for secretory response to anaphylatoxins. Consistent with this idea, proneness for HSRs is known to correlate with the presence of other allergies, i.e., with atopic constitution. Hence, the risk of
CARPA may be highest in those atopic subjects who are also sensitive to C activation.

5. Role of C activation in HSRs to Cremophor EL and other solvent systems containing amphiphilic emulsifiers

The third group of intravenous agents causing CARPA is characterized by the presence of an amphiphilic emulsifier in the infusion liquid, such as the semisynthetic Cremophor EL (CrEL) emulphor (Blum et al., 1979; O’Dwyer and Weiss, 1984; Athanassiou et al., 1988) or synthetic block copolymers, such as poloxamer 188.

5.1. Cremophor EL

Cremophor EL has been used to solubilize many water insoluble drugs, including paclitaxel (Taxol), cyclosporine, the antineoplastic agents Teniposide, Echinomycin and Didemnin E, the anaesthetic agents propanidid and althesin, steroids and vitamins (A, D, E, K) (Lassus et al., 1985). It is a non-ionic detergent, a complex mixture of unmodified castor (ricinus) oil and a large variety of polyethylene glycols and amphiphilic emulsifiers

Patients were classified into four groups according to the concurrent presence (+) or absence (−) of HSR and C reactivity, as follows: true positive (tp: HSR+, C+), false positive (fp: HSR−, C+), true negative (tn: HSR−, C−) and false negative (fn: HSR+, C−). In addition, laboratory reactors were stratified to three categories on the basis of 10 min SC5b-9 values, as specified in column 1. The 0.98 and 1.96 g/mL cut-off values represent two- and four-times the upper limit of normal SC5b-9 levels (0.49 µg/mL), respectively, and were chosen arbitrarily. The sensitivity, specificity and positive and predictive values of the SC5b-9 assay with regard to HSRs were computed as described (Chanan-Khan et al., 2003).

<table>
<thead>
<tr>
<th>SC5b-9 (µg/mL)</th>
<th>Sensitivity (tp/(tp + fn))</th>
<th>Specificity (tn/(fp + tn))</th>
<th>Positive predictive value (tp/(tp + fp))</th>
<th>Negative predictive value (tn/(fn + tn))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant increase* (SC5b-9, no limit)</td>
<td>0.92</td>
<td>0.44</td>
<td>0.57</td>
<td>0.88</td>
</tr>
<tr>
<td>Significant increase* SC5b-9 ≤ 0.98</td>
<td>0.83</td>
<td>0.54</td>
<td>0.45</td>
<td>0.88</td>
</tr>
<tr>
<td>0.98 ≤ SC5b-9 ≤ 1.96 (≤ 2× normal)</td>
<td>0.80</td>
<td>0.70</td>
<td>0.57</td>
<td>0.88</td>
</tr>
<tr>
<td>SC5b-9 ≥ 1.96 (≥ 4× normal)</td>
<td>0.75</td>
<td>1.00</td>
<td>1.00</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Patients were classified into four groups according to the concurrent presence (+) or absence (−) of HSR and C reactivity, as follows: true positive (tp: HSR+, C+), false positive (fp: HSR−, C+), true negative (tn: HSR−, C−) and false negative (fn: HSR+, C−). In addition, laboratory reactors were stratified to three categories on the basis of 10 min SC5b-9 values, as specified in column 1. The 0.98 and 1.96 g/mL cut-off values represent two- and four-times the upper limit of normal SC5b-9 levels (0.49 µg/mL), respectively, and were chosen arbitrarily. The sensitivity, specificity and positive and predictive values of the SC5b-9 assay with regard to HSRs were computed as described (Chanan-Khan et al., 2003).

* Significant increase refers to significant ($P < 0.05$) increase of 10 min SC5b-9 relative to baseline. Reproduced from (Chanan-Khan et al., 2003) with permission.
Abraxane™, an albumin nanoparticle-based paclitaxel formulation, was reported to cause less HSRs compared to traditional Taxol, despite the fact that it was given to patients without steroid and antihistamine premedication, at 50% higher dose and shorter infusion time (Garber, 2004; Sparreboom et al., 2005).

5.1.1. Complement activation as underlying cause of CrEL toxicity

The concept that C activation by CrEL would underlie HSRs to Taxol was based on the demonstration that CrEL fully accounted for C activation by Taxol in vitro (Szebeni et al., 1998). Both Taxol and an equivalent amount of CrEL caused significant elevation of SC5b-9 and Bb in the sera of normal as well as cancer patients following incubation with therapeutically relevant concentrations. This C activation could be inhibited by soluble C receptor type I (Szebeni et al., 1998). As for the mechanism of C activation by CrEL, we considered that CrEL is a non-ionic emulsifier consisting of a mixture of amphiphilic molecules that form micelles in water (Kessel, 1992; Nerurkar et al., 1997; Trissel, 1997).

Micelles are multimolecular aggregates in the nanometer size range, and those formed from amphiphilic polymers usually appear as spherical “core-shell” structures with a dense nucleus surrounded by a less electrodense shell. The size range, and those formed from amphiphilic polymers (Kwon, 2003). Thus, micelles represent a particulate substance unprotected by surface-bound C regulatory proteins (e.g., CR1, DAF, MCP), therefore satisfying two basic conditions for becoming C activator (Liszewski and Atkinson, 1993). It was, however, unclear whether micelles are present in Taxol solutions under the conditions of infusion therapy, and even if the answer is yes, whether they could cause C activation in vivo?

To address the former question, we utilized various physicochemical and imaging techniques to explore and characterize particles in clinically relevant aqueous solutions of Taxol (Szebeni et al., 2001). As shown in Fig. 4A and B, cryo-transmission electron microscopy (Cryo-TEM) showed 8–20 nm spherical structures in aqueous solutions of Taxol mimicking the drug infused in patients, typical “core-shell”, also called “star” micelles that are formed from amphiphilic polymers (Kwon, 2003). As for the question, whether these micelles can cause C activation, we demonstrated that filtration of aqueous solutions of Taxol or pure CrEL via 30kDa cutoff filters eliminated, while the filter retentate restored the potentiating effect of these agents on SC5b-9 formation in human serum (Szebeni et al., 2001). Thus, the effect was due to particles with MW > 30kDa, which is consistent with a causal role of micelles.

However, there was still a problem with the implication of micelles in C activation, considering that their dimension is comparable to the classical and/or alternative pathway C3 convertases (for example, C3bBb is about 14 nm × 8 nm (Smith et al., 1982). Specifically, the surface of even the largest, ~20 nm micelle appears to be too small to allow deposition of C3 convertases, at least as it occurs in the case of C activating cell membranes and other surfaces. A possible solution to this puzzle was provided by our observation (Szebeni et al., 2001) that CrEL micelles underwent massive structural transformation in human plasma, forming microdroplets of varying size up to about 300 nm (Fig. 4C and D). Although we had no experimental data supporting the claim that these microdroplets were in fact formed from CrEL micelles, a previous study by Kessel et al. (1995) provided strong indirect support for this proposition. Namely, in studying the effect of Taxol on plasma lipoproteins in cancer patients, these authors noted an increase in size, as well as a decrease in the electrophoretic mobility of HDL and/or LDL relative to pre-treatment values. They also noted that the originally sharp HDL and LDL bands became smeared, and that a new, highly sudanophilic band was formed which slightly migrated towards the cathode (Kessel et al., 1995). Clearly, CrEL and plasma lipoproteins underwent substantial interaction, which is not surprising in light of the fact that CrEL is a mixture of lipids. In particular, the above data suggest incorporation of CrEL lipids into HDL and LDL, as well as the formation of positively charged particles from apolar molecules in CrEL that did not associate with lipoproteins. Based on these data we suggested that some of the dense, small structures in our cryo-TEM image of human plasma incubated with Taxol (Fig. 4C and D) are CrEL enriched, enlarged lipoproteins, whereas the large lipid droplets may correspond to the newly formed structures composed of hydrophobic CrEL molecules with some charged amphiphilic components at the water–oil interface.

As for the question, how these newly formed CrEL particles in blood activate C, one possibility is that they bind C3 in a fashion similar to that described for the nonionic block copolymer surfactants, L101 and L121 (Szebeni et al., 1998). The latter particles were shown to activate C via the alternative pathway, due to the binding of C3 to their hydrophobic adhesive surface (Hunter and Bennett, 1984, 1987; Hunter et al., 1994). In strong support of this proposal, the length of the hydrophobic (polyethoxylated) chains in many amphiphilic molecules in CrEL is remarkably similar to those found in L101.
Fig. 4. Cryo-TEM images of vitrified specimens of Cremophor EL in saline (PBS) and in human serum. (A) Taxol vial-equivalent Cremophor EL/ethanol stock solution was diluted 10-fold in PBS. The dark spots represent “star” micelles, schematically depicted in the insert. (B) Larger amplification of Cremophor EL micelles. (C) Vitrified specimens of a normal human serum, demonstrating some lipoprotein particles in the chylomicron size range. (D) Cremophor EL was incubated with the same serum for 10 min at 37°C, leading to the formation of numerous particles of varying size. Reproduced from (Szebeni et al., 2001) with permission.

(Szebeni et al., 1998). Furthermore, positively charged particles (liposomes) were reported to induce C activation via the alternative pathway (Chonn et al., 1991).

5.2. Synthetic amphiphilic polymers

Just as the semisynthetic emulsifier molecules discussed above, synthetic amphiphilic polymers have also been used as solvent systems for water insoluble drugs. Some of these polymers have also been used as vaccine adjuvants or as pharmacokinetics-modifier drug conjugates. Examples for synthetic amphiphilic polymers include poloxamers, poloxamines, other copolymers of hydrophilic and hydrophobic blocks, such as polyoxyethylene, polyoxypropylene and polyethyleneglycols (PEG) attached to phospholipids or to low molecular
the phospholipid composition, size, charge and encapsulation was variably present or absent depending on detailed below. With liposomes other than MLV the presence of zymosan caused major hemodynamic changes as cholesterol, 45:5:50 mole ratios) or equal amounts of phosphatidylcholine, dimyristoylphosphatidylglycerol and lamellar vesicles (MLV, consisting of dimyristoylphosphatidylcholine, dimyristoylphosphatidylglycerol and cholesterol, 45:5:50 mole ratios) or equal amounts of nonpolymeric contaminants in the preparation, such as organic volatiles (acetaldehyde and propionaldehyde). C activation was triggered at submicellar concentrations of the polymer and was partially due to the presence of double bonds therein. Consistent with the idea that an interaction with plasma lipoproteins plays a key role in polymer-induced C activation, quasi-elastic light scattering established major changes in lipoprotein size following the addition of poloxamer to plasma (Moghimi et al., 2004). However, poloxamer-induced rise in SClb9 was significantly suppressed when serum HDL and LDL cholesterol levels were increased above normal levels, suggesting that lipoprotein binding can impact C activation by poloxamer in a complex, dose-related fashion (Moghimi et al., 2004).

6. Animal models of CARPA

6.1. Porcine model

Swine are particularly sensitive for liposome-induced cardiopulmonary distress, a feature that may be related to the presence of pulmonary intravascular macrophages in this, as well as in other ungulate (Artiodactyla) species (Winkler, 1988). Over the past 6 years or so, we have injected a total of 105 pigs with various liposomes. Without exception, minute amounts (5–10 mg) of multilamellar vesicles (MLV, consisting of dimyristoylphosphatidylcholine, dimyristoylphosphatidylglycerol and cholesterol, 45:5:50 mole ratios) or equal amounts of zymosan caused major hemodynamic changes as detailed below. With liposomes other than MLV the reaction was variably present or absent depending on the phospholipid composition, size, charge and encapsulated material within the vesicles (Szebeni et al., 1999, 2000a,b, 2002). Interestingly, CrEL did not induce cardiopulmonary distress in pigs even in large (hundreds of milliliters) amounts (unpublished observations). We hypothesize that this unresponsiveness could be due to atypical of ineffective alternative pathway activation (by CrEL) in pigs.

The hemodynamic changes caused by liposomes in pigs include massive rises in pulmonary arterial pressure (PAP) with declines of systemic arterial pressure, cardiac output and left ventricular end-diastolic pressure (Szebeni et al., 1999, 2000a,b). The hemodynamic changes were associated with massive, although transient ECG alterations including tachycardia, bradycardia, arrhythmia, ST segment and T wave changes, ventricular fibrillation and cardiac arrest, all attesting to severe myocardial ischemia and consequent functional disturbance (Szebeni et al., 2005).

C activation-related pulmonary hypertension in pigs was highly reproducible, quantitative and specific. The high reproducibility of the reaction is illustrated by the remarkably low variation in the rise of PAP in response to a same dose of liposomes (Szebeni et al., 1999). The quantitative nature of this “large animal bioassay” was shown by the linear relationship between liposome dose and submaximal rises of PAP (Szebeni et al., 1999), whereas its specificity to C activation became evident from the observations that (1) small unilamellar liposomes, which had negligible C activating effect in vitro, also failed to cause hemodynamic changes in vivo (Szebeni et al., 2000a,b) and (2) non-liposomal C activators (zymosan, xenogeneic immunoglobulins) induced pulmonary pressure changes that were indistinguishable from those caused by MLV (Szebeni et al., 1999).

Considering that (1) hypotension is one of the major symptoms of acute HSRs to liposomes in patients; (2) pulmonary hypertension with consequent preload reduction (decrease of left ventricle filling with coronary hypoperfusion) can explain the dyspnea with chest and back pain in man; (3) the ECG changes observed in the pigs mimic the cardiac electric abnormalities reported in HSRs to liposomes (Ambisome) (Aguado et al., 1993); (4) the vasoactive dose of Doxil in pigs in the 0.02–1 mg/kg range corresponds to the dose that triggers HSR in humans (Gabizon and Muggia, 1998), we proposed that pigs provide a sensitive model of those human subjects who display HSR to liposomes (Szebeni et al., 1999, 2000a,b). As mentioned, the US drug regulatory agency (FDA) recommended consideration of C activation among the immune toxicology tests, when necessary (Hastings, 2002).
6.2. Dog model

While the hemodynamic response of dogs to liposomes is less dramatic than that in pigs, dogs do develop pronounced blood cell alterations in response to liposomes or other C activators (unpublished data). Interestingly, dogs also display considerable vegetative neural dysfunction during HSRs (hyperreflexia, diarrhea, vomiting, extensive salivation), a phenomenon that may represent a unique interaction between the immune and neural systems in this species. As for the hemodynamic changes, it is important to point out that dogs are prone for histamine reactions, and can develop major HSRs without involvement of the C system, as seen with C5EL (Lorenz et al., 1977).

7. Clinical testing of CARPA

The above discussed study of Chanan-Khan et al. (2003), wherein the symptoms of Doxil reactions were quantified and correlated with plasma SC5b-9, may provide guidelines and further ideas for future clinical studies testing the CARPA concept. In particular, the findings highlight the need for measuring other C cleavage products in addition to SC5b-9, as the latter is only an indirect measure of C5a production. It cannot be excluded that some clinical reactors with no elevated SC5b-9, who were considered “false negative”, actually produced increased amounts of C3a (and/or C5a). Possibly useful is the demonstration of increased susceptibility to C activation proceeds on a time scale of minutes, the time window of blood sampling and in vitro basophil activation should cover the first few minutes.

Apart from better understanding the pathomechanism of HSRs, the CARPA concept may represent a step forward in solving a major dilemma in theoretical immunology: the classification of HSRs. Gell and Coomb’s system of four categories (types I–IV) has serious limitations, including the fact that pseudoallergy cannot be fitted in any of the four types of HSRs. However, no consensus has been reached to date, how to replace this classification. Descotes and Choquet-Kastylevsky (2001) proposed the use of three major types, namely, pseudoallergy, immunoglobulin-mediated and cell-mediated HSRs. Aronson and Ferner (2003) suggested to specify and graphically characterize HSRs according to their time course, susceptibility and dose dependence. The author or this review (Szebeni, 2001) laid out a functional categorization that differentiates acute (Type I) HSRs according to the underlying mechanism of mast cell and basophil release reactions. The scheme differentiates these major subclasses: (1) direct cell activation and (2) receptor-mediated activation, with the latter category encompassing three subcategories: (a) IgE-triggered and FcεRI receptor mediated, (b) anaphylatoxin-triggered and C3a/C5a receptor-mediated “CARPA” and (c) mixed type reactions, triggered by both IgE and anaphylatoxins (Fig. 5). This classification covers many previously uncategorized, C-mediated reactions that arise upon the
use of extracorporeal circuits, RCM and various liposomal and micellar carriers of intravenous drugs.

References


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