ADVERSE IMMUNE EFFECTS OF LIPOSOMES: IMMUNOGENICITY AND IMMUNE SUPPRESSION

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Abstract: Some therapeutically relevant liposomes are recognized by the immune system as foreign, and the resulting innate or specific immune response can be adverse to the host. The innate response involves activation of the complement system which, via liberation of anaphylatoxins (C5a, C3a), underlies an acute hypersensitivity syndrome called C activation-related pseudoallergy (CARPA). CARPA represents a potential barrier to the clinical use of reactogenic liposomes in cardiac patients, as a main manifestation of C activation in the body is cardiopulmonary distress. The adverse immune response to liposomes involving specific immunity is exemplified by PEGylated nano-liposome induced transient IgM production, which causes accelerated blood clearance (ABC). This manuscript highlights some common and specific causes of adverse immune effects of liposomes.

Keywords: Immunogenicity, Immune Suppression

Introduction
The absence of adverse immune effects, a unique plus point of simple liposomes, will probably become the exception rather than the rule. Liposomal immunology will widen its present focus in the employment of liposomes as vaccines, to mapping an uncharted network of hypersensitivity and immunogenicity reaction pathways. As the safety of medicinal nanoparticles comes more to the forefront, reactogenicity and immunogenicity testing may join the list of toxicity assays required by regulatory agencies. A deeper insight into the fine immunomodulatory effects of complex liposomes may also lead to a need to revise our views on immune suppression, as is the case with Doxil [1].

Types and features of Immune Responses to Liposomes
Immune responses to liposomes can be stimulatory or inhibitory, weak, moderate or
severe, all with a broad variation in their time of onset and duration (Table 1).

**Table 1. Variations of liposome-induced immune changes according to the time of onset and duration.**

<table>
<thead>
<tr>
<th>Type of change</th>
<th>Time of onset</th>
<th>Duration</th>
<th>Example &amp; Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation</td>
<td>Immediate (within seconds to minutes)</td>
<td>Minutes to hours</td>
<td>Hypersensitivity (infusion) reactions caused by liposomes [2,3,4]</td>
</tr>
<tr>
<td></td>
<td>Delayed (within hours)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Late (within days to months)</td>
<td>Weeks to years</td>
<td>Immunity to liposomal antigens, e.g. influenza or hepatitis B [5,6]</td>
</tr>
<tr>
<td>Inhibition</td>
<td>Short-term</td>
<td>Hours to days</td>
<td>Liposomal alendronate [7]</td>
</tr>
<tr>
<td></td>
<td>Long-term</td>
<td>Days to months</td>
<td>Doxil induced immunosuppression[8,9,10].</td>
</tr>
</tbody>
</table>

**General Causes Behind Immune Recognition of Liposomes**

Among many structural features, two stand out as fundamental reasons for immune recognition of liposome(11) the diameter of vesicles, roughly in the 50-200 nm range and (8) absence of cell membrane structures that normally prevent host cells from immune recognition. This overlap, however, may not be simple coincidence, but rather the consequence of those common physicochemical rules and forces that force bilayer membranes to form vesicles in live systems and in test tubes; in fact, liposomes are considered as the archetype bilayer membrane that enabled cellular evolution over millions of years.

Liposomes also resemble ectosomes, i.e. membrane vesicles detached from cells, as well as most other ecto-organelles and cellular debris that form upon cell death. Nanobacteria, the smallest self-replicating pathogens are also in the liposome size range (100-200 nm) [12]. In essence, liposomes mimic the size and shape of pathogenic microbes and some subcellular structures against which nature developed strong eliminatory mechanisms via humoral and cellular immune responses.

**Immunogenicity of Liposomes**

Being built from natural phospholipids, liposomes are generally not immunogenic. This statement is in apparent conflict with the intense ongoing R&D of liposomal vaccines, however, it should not be forgotten that these vaccines include protein or lipid antigens and adjuvants and mediators, such as lipid A, muramyl dipeptide and its derivatives, interleukin-1, and interleukin-2, in addition to the phospholipid bilayer, which also acts as an adjuvant by its own right [13]. When adjuvants are used, such as lipid A, specific antibodies are induced against all liposome components, including structural (phospho)lipids, cholesterol and squalene, a cholesterol precursor triterpene with a barely distinguishable antigenic epitope [14,15,16].

As for the underlying cause of the intrinsic adjuvant capability of phospholipid bilayers, promoting specific immune response to liposomal antigens and non-antigenic lipid components (without additional adjuvant), the “array theory” [17] provides a likely
explanation. Adapting this theory to the special case of immunogenic non-vaccine liposomes, it can be proposed that because of their similarity to viruses, liposomes may present their surface conjugates or protruding repetitive surface elements to APC and other immune cells (monocyte/macrophages, dendritic cells, B lymphocytes and mast cells) in the form of an array which resembles the regular and symmetric spatial arrangement of viral capsid glycolipids and glycoproteins, for which the so-called “pattern recognition receptors” (e.g., LPS and Toll-like receptors, TLRs) on the above cells readily react, generating innate and subsequent specific immune responses. Originally TLRs recognize molecule arrays that are broadly shared by pathogens (called pathogen-associated molecular patterns, PAMPs, such as LPS, lipoproteins, lipopeptides, flagellin, double-stranded RNA or the unmethylated CpG islands of bacterial and viral DNA), however, “liposomal arrays” also trigger “danger” signaling within these cells despite the absence of PAMPs, which ultimately leads to antibody production against the “pseudo-PAMPs” and their phospholipid support. The resultant immune response may or may not differ from a standard immune response to vaccines, depending on the pathway of immune activation.

**Immune Suppression by Liposomes**

Postoperative bacterial infections are common despite prophylactic administration of antibiotics. The wide-spread use of antibiotics in patients has contributed to the emergence of multiresistant bacteria. A restricted use of antibiotics must be followed in most clinical situations. In surgical patients there are several reasons for an altered microbial flora in the gut in combination with an altered barrier function leading to an enhanced inflammatory response to surgery [18]. Infliximab is a chimeric monoclonal antibody, belonging to the class of anti-tumor necrosis factor-α (TNF-α) agents, approved for the treatment of psoriasis and psoriatic arthritis. Drugs of this class are known to be associated with an infective risk, probably because they interfere with inflammatory and immune response at different levels [19].

Infectious complications are a major cause of morbidity and mortality from dose-intensive cancer chemotherapy. In spite of the importance of intestinal bacteria translocation in these infections, information about the effect of high-dose chemotherapy on gut mucosal immunity is minimal [20]. There are no data concerning the significance of allergen specific nasal challenge to latex (ASNCL) in the pediatric population and the effect of mometasone furoate nasal spray (MFNS), topical corticosteroid exerting a potent anti-inflammatory activity in children with latex allergic rhinitis [21].

Phenothiazines, which are also employed for premedication, are known to have an inhibitory effect on the cell-mediated immunity. Therefore, the effect was studied in nine patients of the most commonly used atropine-pethidine premedication on the leucocyte and differential count, the number of T-and B-lymphocytes and the lymphocyte transformation by phytohaemagglutinin (PHA), Concanavalin A (ConA), pokeweed mitogen (PWM) and purified protein derivative of tuberculin (PPD) in cultures of separated lymphocytes and of whole blood. The premedication increased the proportion and absolute number of surface immunoglobulin-positive (B) lymphocytes in the peripheral blood and reduced the PHA and PPD responses in whole blood cultures. These vague changes in the immune response after premedication are of no importance in clinical work [22].

It is well known that liposomes are taken up mainly by cells of the reticuloendothelial system (RES) in the liver, spleen, bone marrow and elsewhere, which cells are also part of the nonspecific, innate immune system. Therefore, it has been asked for a long time whether macrophage saturation by liposomes, leading to immune suppression, could be a problem, a potential risk for infection? There is ample evidence that clinically applied doses of non-cytotoxic liposomes generally do not cause immune suppression, at least not major, clinically important blockage of macrophage function. However, the situation is different with anticancer liposomes that contain cytostatic drugs,
which may cause more or less immunosuppression.

Conclusions

Immunosafety is a key issue in current R&D of nanomedicines, including liposomes. One main problem in this field lies in the complexity and individual variation of the immune system, which, when faced with increasingly complex nanomedicines, will also give increasingly complex responses. The immune toxicology of nanomedicines is largely unexplored at a broad intersection of nanotechnology, immunology and pharmacology. Hopefully it is not too far in the future that we can equip liposomes and other drug carrier nanosystems with immune evasive capabilities, and/or “teach” the immune system to distinguish these marvels of nanotechnology from harmful microbes.

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