



Opinion

Anti-Poly(ethylene glycol) (PEG) Antibodies: From Where Are We Coming and Where Are We Going

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Abstract: PEGylation technology confers stability and modulates the biological performance of a broad range of preclinical and clinical nanopharmaceuticals. However, the emerging PEG immunogenicity in the general population is thought to impact the efficacy and safety of PEGylated medicines. Despite this, the clinical significance of PEG immunogenicity is still not clear and remains debatable. By considering the strategic importance of the PEGylation technology in nanopharmaceutical engineering, we raise a number of critical questions and briefly discuss gaps in the knowledge of PEG immunogenicity and its clinical significance.

Keywords: antibodies; complement system; immunogenicity; poly(ethylene glycol)



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1. Introduction

Grafting of poly(ethylene glycol) (PEG) to peptides, proteins, nucleic acids, and nanopharmaceuticals is a widely established strategy to improve stability and pharmacokinetic properties [1,2]. However, it is the emerging high (and possibly increasing) prevalence of anti-PEG antibodies of IgG and IgM class in the general population which is anticipated to impact treatment efficacy, for instance, by triggering premature clearance and/or modulating/damaging the integrity of PEGylated medicines, such as PEGylated liposomal products, and inducing adverse effects (e.g., anaphylaxis) [3–5]. While the majority of these predictions are extrapolated from animal studies, the relevance of observations in animal models to humans is still not clear and remains debatable [6]. There are, however, some studies indicating that anti-PEG antibodies are boosted in humans by the SARS-CoV-2 lipid nanoparticle mRNA vaccine which contain PEGylated lipids and could have been responsible for rare episodes of anaphylactic reactions, possibly through complement activation [7–9], while there are other studies that do not support a role of pre-existing anti-PEG antibodies, including anti-PEG IgE and IgG, for post-lipid nanoparticle mRNA COVID-19 vaccine anaphylaxis [10]. The differences in assay conditions, case–patient sample size, and the possibility that some of cases were not anaphylaxis might explain the aforementioned discrepancies. We still await conclusive evidence to support that anti-PEG antibodies trigger anaphylaxis in humans [11]. Thus, comprehensive studies are needed to purify and characterize these antibodies and delineate the differences in their properties among different individuals. Notwithstanding the large body of knowledge, it is useful, perhaps, to identify the gaps in the knowledge to allow us to move the field forward and put anti-PEG antibodies in the perspective of other developments within the pharmaceutical field.

2. Immunogenicity of Pharmaceuticals

It is well recognized that the immunogenicity of pharmaceuticals (small molecules, biologics, cell-based products) presents a major challenge for patients' health and pharmaceutical development [12]. Preexisting antibodies or de novo elicited antibodies against pharmaceuticals are undesirable and can lead to deleterious consequences, from diminished efficacy to outright toxicity and anaphylactic response. Thus, anti-drug antibodies against small molecules induced via the hapten mechanism have been known for a while and can lead to decreased efficacy upon reinjection and anaphylaxis (e.g., penicillin) [13]. Antibodies against biologics are a well-known issue that can lead to infusion reactions and lethal outcomes, for example, against asparaginase [14] or early generations of monoclonal antibodies that contain mouse sequences [12]. There are several courses of action to reduce pharmaceuticals' immunogenicity and improve their safety, for example, by reengineering and replacing highly antigenic domains of antibodies with human sequences. In the case of cell-based products, autologous products are preferred, whenever possible, or CRISPR-edited off-the-shelf products that are devoid of MHC class II molecules (as well as other potentially immunogenic moieties) are used.

3. Revisiting PEG Immunogenicity

Considering the strategic importance of the PEGylation technology versus the emerging PEG immunogenicity, there are several unanswered questions. Anti-PEG antibodies are often measured by direct binding assays using beads or ELISA plates [3]. Still, variations in assay conditions and especially assay cut-off criteria might explain the wide range of positive frequencies reported for anti-PEG antibodies [10,15]. Accordingly, the standardization and harmonization of anti-PEG assays are necessary and discussed elsewhere [15].

Furthermore, we still lack sufficient knowledge of the binding specificity of anti-PEG antibodies. Thus, how efficiently do anti-PEG antibodies bind to nanopharmaceuticals in the blood (or in neat serum), especially with anti-PEG antibodies that exhibit low affinity for PEG? Even in the presence of high titer specific antibodies, are these going to be the only antibodies that bind to nanoparticles in the blood (as opposed to the highly diluted conditions reported in anti-PEG assays)? Can other antibodies, such as anti-cholesterol, anti-squalene, and anti-phospholipid antibodies, bind PEG and influence readouts? On exposure to blood, nanoparticle surfaces, including PEGylated nanomaterials, are enriched with blood proteins [16]. We wonder what the percentage of anti-PEG antibodies among all other antibodies that bind is in a typical nanoparticle proteome. Our studies [16] suggest that most of the antibodies bind to the nanoparticle proteome rather than to the pristine surface. Considering the above, the ELISA procedures should avoid PEG-containing surfactants as diluents and be validated through careful competitive inhibition studies, where the choice of competition reagent should closely resemble the PEG characteristics of the PEGylated drug used or preferentially be the same type as the PEGylated drug used [15].

Does measuring anti-PEG antibody titers have a clinical significance? Measuring anti-drug antibodies is not practical in most cases, as there is no definite correlation (only association) with side effects and toxicities. Furthermore, even with very established medicines that are intended to elicit an antibody response, such as COVID-19 vaccines, measurements of antibody titers as an indicator of vaccine efficacy (or, for that matter, any other vaccine) are rarely done and cannot serve as a reliable metric of vaccine efficacy. Therefore, such a test is left to the discretion of the consumers and is not commonly paid for by medical providers. In this environment, it is hard to envision that measuring anti-PEG antibodies will be widely adopted in the clinic, except for the use of one of the research biomarkers related to toxicity/efficacy in early clinical trials.

In contrast, when it comes to disease diagnostics, the measurement of antibodies (e.g., anti-nuclear antibodies in lupus or antiphospholipid antibodies) is commonly accepted because these antibodies are valuable in assessing disease severity. Measuring anti-PEG antibody titers makes sense only if their clinical significance is clear [15]. Recent reports that

ambiguously correlate these antibodies with hypersensitivity reactions to nanomedicines, such as the COVID-19 lipid nanoparticle mRNA vaccines [17], are premature, and the jury is still out on that front. Given the extremely rare incidence of these reactions, it is hard to envision a companion test before administering these vaccines. It is also noted that there was no boosting of anti-PEG IgG and IgM antibodies in patients receiving PEGylated liposomal doxorubicin [15]; instead, this process resulted in a decrease in titers in seropositive patients [15]. This observation is in line with the hypothesis of the induced damage to the anti-PEG antibody-producing immune compartment, such as the splenic B cells, by the PEGylated medicine [18].

Notwithstanding, one intriguing question that could perhaps shed light on the significance of anti-PEG antibodies is why, with such a high prevalence, there are almost no known allergies or hypersensitivities to PEG-containing cosmetic and hygiene and healthcare products. Since we are all exposed to PEGylated products from early childhood, is it possible that we develop some form of immunological tolerance? It is also plausible that these antibodies are present at concentrations and/or affinities with little or no clinical impact.

The antibody's effector functions are primarily mediated by binding to Fc-receptors or through complement activation, and complement activation, when uncontrolled, might turn its destructive actions against the host [19]. Thus, some classes of anti-PEG antibodies might induce hypersensitivity to PEGylated medicines in humans through interaction with Fcγ receptors which have different affinities, signaling routes and patterns of expression on innate immune cells [20], a possibility that has received limited attention to date [21]. Last but not foremost, do anti-PEG antibodies, or any "anti-nanoparticle" antibodies in general, predict complement activation? Our recent data on SARS-CoV-2-like nanoparticles decorated with Receptor Binding Domain (RBD) antibodies clearly show that there is a positive trend, i.e., vaccinated donors with high levels of anti-RBD antibodies generally lead to high complement activation [22]. However, this trend is not absolute and, overall, the level of complement activation by RBD-decorated nanoparticles in seropositive donors is similar to the non-vaccinated (pre-pandemic) cohort. Another unexplored aspect of anti-PEG antibody-mediated complement activation could be enhanced immune uptake, and studies on complement-dependent immune uptake in whole lepirudin blood could prove invaluable. Our previous work demonstrated the clear effect of complement opsonization on the uptake by blood leukocytes (predominantly by monocytes and neutrophils, which are the prime phagocytic cells) [22,23]. This study highlighted the highly variable but, in most cases, complement-dependent uptake of iron oxide nanoparticles of different compositions [23].

Furthermore, investigations with RBD-decorated iron oxide nanoparticles showed that, while most of the donors' leukocytes exhibited complement-dependent uptake, only a few donors with the highest titers clearly demonstrated the effect of anti-RBD antibodies on leukocyte uptake of nanoparticles [22]. It must be remembered that the binding of antibodies to pathogens can promote immune uptake via complement-independent processes, and there is also the interplay between binding to the Fc receptors and to the complement receptors on immune cells, including neutrophils and monocytes. Therefore, a crucial question towards addressing the clinical significance could be the following: Does the titer of anti-PEG antibodies affect C3 opsonization and the immune cell uptake, and how variable are these responses in different donor populations (e.g., patients with proinflammatory conditions, acute infections, etc.)? Considering that the interaction of non-specific antibodies with nanoparticles triggers C3 deposition and modulates nanoparticle clearance by leukocytes, it might be more plausible to build a prediction risk based on total antibody binding to a test nanoparticle. Such attempts should further consider the polymorphic nature of key complement proteins (complotype) [24,25], since such genetic mutations can further modulate the effect of the nanosurface-binding antibodies.

Finally, the answers to the questions mentioned above might determine whether PEG alternatives are necessary. Nevertheless, the development of other options, such as

polyoxazolines [26] or the use of albumin as a PEG substitute [27,28], is thriving. Still, each of these systems is expected to have its limitations [29].

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