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Perspective

Are Injection Site Reactions in Monoclonal Antibody Therapies Caused by Polysorbate Excipient Degradants?

Satish K. Singh^{1,*}, Hanns-Christian Mahler¹, Christine Hartman², Craig A. Stark²¹ Lonza Pharma and Biotech, Drug Product Services, Hochbergerstrasse 60A, Basel CH-4057, Switzerland² UnityPoint Cardiology, 1215 Pleasant, Suite 414, Des Moines, Iowa 50309

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ABSTRACT

Injection site reactions (ISRs) and other adverse side effects are commonly observed during therapy with biologics. These hypersensitivity-related side effects can vary from simple rash to life-threatening anaphylactic reaction and may be linked to the immunogenicity of the drug including formation of antidrug antibodies. Reactions can also occur as a consequence of excipients in the product. We report the case of a patient who developed erythematous ISRs to both commercial PCSK9i formulations and had to go off therapy even though efficacy was not impacted. Skin testing showed that the patient was reacting to the polysorbates. Polysorbates are added to stabilize the biotherapeutic. Polysorbates can also activate complement and lead to a range of acute hypersensitivity and systemic immunostimulation reactions. Oxidative degradation products can function as haptens by reacting with proteins at the injection site. Reactive degradation products may even form adducts with the biologic itself, creating a potential neoantigen. Further research is needed to understand the fundamental causes of ISRs. It is critical that only the highest quality raw material is used, and proper storage conditions are employed to minimize degradation of polysorbates in the product. Although complete elimination of ISRs is unlikely, all efforts must be made to minimize them.

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Introduction

The past 3 decades have witnessed an explosion in the use of monoclonal antibodies (mAbs) to treat human diseases after they were first used to treat transplant rejection.¹ Initial mAbs were nonhuman (murine or chimeric) resulting in significant immunogenicity and related off-target adverse reactions. As antibody technology has evolved, most mAbs used today are “humanized,” retaining only small segments of nonhuman amino acid sequences or they are “fully human,” retaining no nonhuman amino acid sequences which is expected to lead to a decrease in their intrinsic immunogenicity.² mAbs are now widely used in the treatment of oncologic, autoimmune, and inflammatory disorders. However, despite their “humanization,” mAbs are still associated with intriguing off-target adverse reactions.^{3–5}

Almost all biologics are administered parenterally, with the intravenous (IV), intramuscular (IM), and subcutaneous (SQ) routes being the most common. In general, IV injected drugs achieve brisk peak serum concentrations and become rapidly diluted into the

blood pool and the extracellular space. Drugs administered IM do not achieve the same brisk serum peak concentrations as seen from IV injections. Drugs injected IM are, however, adsorbed more rapidly than SQ injected medications because muscular tissues are more vascular.⁶ SQ injected drugs are retained at the injection site for longer periods of time than those administered either IV or IM, and thus result in an extended release of the drug. This retention time would likely be exaggerated with high concentration viscous mAb solutions. The skin is also an immunological organ.⁷ Subdermal tissue contains significantly more dendritic cells than skeletal muscle but the SQ space contains few of these potent antigen-presenting cells. Dendritic cells are, however, enriched and in a more activated state in the SQ space than in circulation. Cutaneous dendritic cells also migrate to the SQ space on injection, and this migratory potential can drive immunogenicity against the injected protein. Larger proteins (such as mAbs) are primarily absorbed through the lymphatic pathways from the SQ depot and are thus subject to 2 rounds of antigen presentation: first in the region around the injection site and lymphatic transport and then in systemic circulation through the mononuclear phagocytic system or the secondary lymphatic organs such as liver and spleen. The SQ route of administration is thus often considered more likely to elicit an immune response than IV. Although generally true for vaccines or foreign antigens, the evidence is mixed for case of therapeutic

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* Correspondence to: Satish K. Singh (Telephone: +41 79 765 2843; +1 636 448 1458).

E-mail address: satish.singh@lonza.com (S.K. Singh).<https://doi.org/10.1016/j.xphs.2018.07.016>

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proteins that are self or designed to mimic self (see reviews by Fathallah et al.,⁸ Hamuro et al.,⁹ and Turner and Balu-Iyer¹⁰)

Widespread use of biologics in a variety of indications and disorders has also led to increasing and sometimes novel adverse drug reactions. Traditionally, hypersensitivity reactions have been classified into types I–IV.^{11,12} More recently, an alternative classification system of adverse reactions to biologic drugs has been proposed due to differences in the mode of action, metabolism, and immunogenicity of biologic drugs compared with other xenobiotics. This classification is illustrated in Figure 1.^{13–15} Type β or hypersensitivity reactions generally manifest as either an immediate infusion or injection site reactions (ISRs).⁵ These reactions are generally linked to the immunogenicity of the drug. Rapid appearance of hives is likely to be an IgE-mediated phenomenon. Acute infusion reactions without hive formation may arise because of the presence of human antidrug antibodies (HADAs) with subsequent activation of the complement system. Delayed serum sickness reactions usually manifest more than 6 h after drug administration (including vasculitis and nephritis). These reactions may be due to either HADA reactions or cell mediated in etiology. ISRs may also occur to excipients with allergenic potential.^{5,14}

This article summarizes our findings related to a literature-based investigation into the reasons for development of ISRs during monoclonal antibody therapies, stemming from a case with PCSK9i therapy. Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibition is a target in treatment of hypercholesterolemia (CHOL)¹⁶ and 2 fully human mAbs are currently available commercially. Praluent® (alirocumab) and Repatha® (evolocumab) are approved for self-administration biweekly or monthly by the SQ route. Long-term follow-up of patients in clinical trials reports the following incidence for the development of HADA in these products: 0.1% per Repatha® prescribing information and 4.8% per Praluent® prescribing information.^{17,18} (We note that immunogenicity rates should not be compared across products due to differences in sensitivity and specificity of assays and sampling-related variations). Our investigation showed that the patient in this case, who developed ISRs to both products, had a hypersensitivity to polysorbate 80 (PS80). We believe that this is the first literature report of PCSK9i mAb excipient dermal hypersensitivity, specifically a reaction to the pharmaceutical excipient, polysorbate (80 and 20). (This case was reported as an abstract,¹⁹ and a more

detailed medical report is in preparation). A case report was recently published describing the lack of cross-reactivity of an allergy reported in a patient who was receiving Praluent®, who was successfully switched to Repatha® and tolerated Repatha® without any adverse ISR.²⁰ No explanation is given within this report for the ISRs that developed to Praluent®. We propose that immunologic reactions connected to polysorbates may form a basis for the ISRs in this report and of the ISRs associated with many other biologics therapies. Because polysorbates are almost universally used as a key excipient in the formulation of biologics, it is important for practicing physicians and pharmaceutical development scientists to understand the risk profile of this excipient and particularly of its degradation products.

Case Summary

The patient is an 80-year-old female with a history of coronary artery disease with CHOL with a low-density lipoprotein cholesterol (LDLc) of 111 mg/dL. She had never received therapy with either IV immunoglobulin or any other mAb product. She was started on Praluent® 75 mg SQ every 2 weeks. One month later, her LDLc was 41 mg/dL. Three and a half months later, she reported developing an erythematous ISR to Praluent®. Praluent® was stopped, and she was started on Repatha® 140 mg SQ every 2 weeks. Repatha® injections were initially well tolerated. However, 6 months later she developed recurrent erythematous ISRs that became apparent 1–2 h after the administration of Repatha®. She reported that the Repatha® ISRs were similar in appearance to the prior Praluent® ISRs, but now the size of the rash was getting larger. She was instructed to pretreat herself with oral diphenhydramine and to apply topical 1% hydrocortisone cream, 1 h before her next scheduled injection of Repatha®. She returned 2 weeks later, a few hours after taking her injection of Repatha® with a 5 × 7 cm erythematous ISR (Fig. 2; see Disclaimer). Diphenhydramine and topical steroids were continued. Re-inspection of the ISR 24 h later demonstrated persistence of erythema. During this visit, her LDLc was still 43 mg/dL. At the time, she was given a second dose of Repatha® 140 mg SQ into her contralateral thigh. An ISR developed slowly at that injection site as well. Injections of Repatha® were subsequently discontinued despite good control of LDLc.

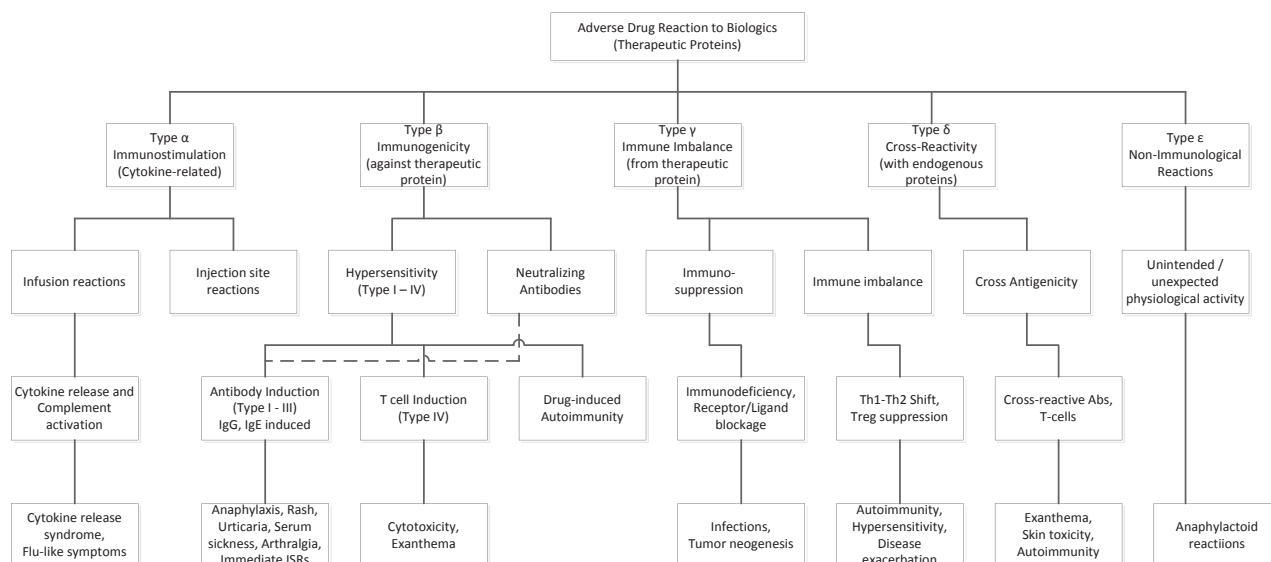


Figure 1. Classification scheme for adverse drug reactions to biologics. Modified from Scherer et al.,¹³ with permission.



Figure 2. Injection site reaction. Photograph used with permission (see Disclaimer).

Analysis and Results

Evolocumab is a fully human IgG2- λ mAb.²¹ Repatha® is a sterile, preservative-free, clear to opalescent, colorless to pale yellow solution for SQ administration. Each dose (volume = 1 mL) contains evolocumab (140 mg/mL), acetate (1.2 mg/mL), polysorbate 80 (0.1 mg/mL), proline (25 mg/mL) in water for injection at pH 5.0.¹⁸ (A change in formulation to the commercial version was made late in phase 3 trials. Preclinical to early phase 3 studies were conducted with a 70 mg/mL product containing acetate, sucrose, and polysorbate 20).²² The European Medicine Agency review of clinical outcomes of 6026 patients who had received Repatha® indicated that 15 patients (0.2%) developed HADA following at least 1 injection of Repatha®.²² In the FOURIER Trial, 43 of 13,784 patients (0.3%) receiving Repatha® developed HADA.²³ In both the groups of patients receiving Repatha® long term, no individuals with a documented HADA ever developed a neutralizing antibody or reported any other significant adverse event (SAE). In homozygous familial CHOL studies, 1 subject (2.4%) discontinued treatment due to a grade 3 adverse event (AE) of rash.^{21,22} ISRs were slightly more common in patients exposed to the active evolocumab (2.1%) versus placebo (1.6%) in the FOURIER trial.²³

Alirocumab is a fully human IgG1- κ mAb.²⁴ Praluent® is a sterile, preservative-free, clear, colorless to pale yellow solution for SQ administration. Each dose (volume = 1 mL) contains 75 or 150 mg/mL alicumab with histidine (6 or 8 mM), polysorbate 20 (0.1 mg/mL), sucrose (100 mg/mL) in Water for Injection at pH 6.0.¹⁷ HADA development was 4.8% (vs. 0.6% in the control group) with 1.2% being neutralizing, but classified as being transient. The incidence of SAEs was approximately similar in HADA positive patients irrespective of titer. The incidence of AEs was slightly lower (67% vs. 76%) and similar for allergic AEs (8.3% vs. 8.2%), in the HADA-negative population. The European Public Assessment Report states that the rate of ISRs was comparable to that of HADA-positive and HADA-negative patients (although this was based on the limited number of patients with neutralizing antibodies).²⁵ However, in data from 3 combined phase 3 trials, local ISRs occurred at a higher frequency (10.2%) in HADA-positive patients compared with 5.9% in HADA-negative patients. In the ODYSSEY trial, among alicumab-treated patients, those with persistent HADA had higher frequency of ISRs (15.9%) compared with transiently positive (11.6%) or negative (5.9%) HADA patients.²⁶ Overall local ISRs were

more frequent in the active treated group versus control group (7.2% vs. 5.1%¹⁷; 6.7% vs. 4.8%²⁵; 6.1% vs. 4.1%²⁶). Overall incidence of treatment associated AEs leading to discontinuation of investigational product was similar between active and placebo groups (5.3% vs. 5.1% respectively). Incidence of ISRs leading to discontinuation of investigational product was 0.2% (5/2476) and 0.3% (4/1276) in the active and placebo groups. Symptoms associated with local ISRs included erythema/redness (2.9%), swelling (2.3%), pain (1.9%), and hematoma (0.3%).²⁵

Patient experience taken in context with literature information leads us to decipher the likely etiology of the ISRs in this case. First, based on one of the authors' own personal experience treating patients with PCSK9i to date, ISRs of erythema have been seen in multiple other patients. No patient has ever reported developing an ISR at the time of the first injection. Rather, ISRs have appeared after the first month of therapy. This suggests an ISR that is acquired by sensitization, similar to a classic "Arthus reaction."²⁷

Second, the incidence of HADA development in clinical trials with the PCSK9i mAbs was small (~0.1%–0.3% and 4.8%). This suggests that the risk of any particular patient developing HADAs against both alicumab and evolocumab should be negligible. There was no loss of efficacy of LDLc lowering, thereby excluding the possibility of neutralizing antibodies. There are no other published reports describing ISRs in patients who have received both Praluent® and Repatha®. However, an association between development of HADA and ISRs was seen in Praluent® clinical trials (as discussed previously), and also in the SPIRE program with bocicizumab (which was under development as a PCSK9i).²⁸ SPIRE patients developed HADA at a high rate (48% at 1 year with 29% neutralizing). Of patients in the SPIRE active treatment group, 12.7% also developed erythematous ISRs versus 1.6% of placebo-treated patients. This is a much higher incidence of both HADA and ISR than what was seen in the clinical trials with Praluent® and Repatha® and may have been one of the causes for termination of the program. (Note that bocicizumab is a humanized antibody as opposed to the fully human evolocumab and alicumab under discussion here, and may have been one factor in increasing the propensity for HADA development).^{28,29} Taken together, these suggest an IgG-mediated type β hypersensitivity reaction with possible development of non-neutralizing HADA (Fig. 1).

Third, although evolocumab (and generally any SQ-injected high concentration mAb) reaches peak serum concentrations ~5–10 days after SQ administration, serum concentrations of PCSK9 become undetectable within hours following the SQ administration of the mAb.³⁰ If the inflammatory rash occurred because evolocumab-PCSK9 enzyme complexes formed at the initial administration of Repatha®, which could then activate macrophages and complement, then the re-administration of Repatha® 24 h later should not be able to demonstrate the appearance of any ISRs. This is because a certain stoichiometry of antigen (PCSK9 in this case) and antibody (mAb) would be needed to produce pathologic immune complexes that efficiently activate complement.^{31,32} However, because ISRs did occur with the 24-h re-injection of Repatha®, at a body location which had not been used by this patient before for any previous mAb injections, it may be surmised that the rash was not due to evolocumab-PCSK9 enzyme immune complex formation. The sensitivity that was being demonstrated was a generalized diffuse reaction that now took place within the entire body rather than a localized area of irritation from multiple preceding injections at the same spot on the body. Furthermore, evolocumab is built on an IgG2 platform and should not readily activate complement.³³

Based on the similarity of ISRs of the patient with the 2 different PCSK9i, and the information from the second Repatha® injection, the cause of the ISRs was subsequently hypothesized to be due to a formulation, that is, excipient-related reaction instead of reaction

related to the active agents or their pharmacological effect. Both products contain polysorbates: Praluent[®] contains 0.1 mg/mL polysorbate 20 and Repatha[®] contains 0.1 mg/mL polysorbate 80; the dose volume used was 1 mL SQ biweekly for both products. The patient was subsequently skin tested with 0.1 mL of polysorbate 80 given intradermally (concentration 0.1 mg/mL, pH 5.5, sterile filtered injection prepared by a compounding pharmacy). She started to develop erythema without urticaria or any wheal reaction at the injection site within 15 min which progressively increased in size over 2 h. However, no reaction was observed at the near simultaneous administration of sterile normal saline 0.1 mL on the contralateral forearm at the time of the skin test. The skin test site was re-evaluated the following day and showed almost complete resolution of erythema and induration.

Discussion

Excipients in biologic product formulations are intended to stabilize the active protein from manufacturing until its use by the patient.³⁴ Excipients are designated by the regulatory authorities to be inactive, in that they are not considered to be the primary biologically active ingredient.³⁵ However, this does not necessarily imply that these excipients are always biologically and pharmacologically inert on administration (see e.g., Dantal³⁶). In regulatory filings, the need for and function of each excipient has to be properly justified, and the entire to-be-marketed product is tested in clinical trials for safety and efficacy. New or novel excipients must undergo a rigorous safety assessment before their use.³⁷ However, existing excipients with precedence for use by a proposed administration route and at doses used within the range of successful prior experience are generally assessed only indirectly via the overall safety profile of the product being studied. The polysorbates (PS20 and PS80) fall into this latter category for parenteral injection, because of their long history of usage in such products.

Polysorbates belong to a family of ethoxylated nonionic surfactants and are polyoxyethylene-1,4-sorbitan-monoesters of fatty acids (FAs). PS20 and PS80 utilized in the industry are pharmacopeia compliant and contain heterogeneous mixtures of monoesters, diesters, and triesters of a range of FAs.^{38,39} The main differences between PS20 and PS80 is in the composition of the FAs.^{40,41} The predominant FA in PS20 is lauric acid (C12:0) and in PS80 is oleic acid (C18:1). PS80 has a greater proportion of longer chain and more unsaturated FAs than does PS20. Levels of PS used in biological products generally ranges between 0.001% and 0.04% w/v, although higher levels are also found. Both PS80 and PS20 are frequently used in these products, and current understanding of the mechanisms of their functioning does not suggest any differentiation between them. Use of one or the other polysorbate is often driven by normal practice at the development organization.

Polysorbates (PS80 and PS20) both as a raw material, and in solution after their addition to mAb products, are subject to degradation by oxidation. The oxidation of PS (in the oxyethylene chains and in unsaturated FAs where present) leads to the formation of peroxides which can trigger a series of free-radical mediated auto-oxidation reactions resulting in the formation of FA esters, aldehydes, alkenes, epoxides, ketones, polyethylene glycol esters, and free fatty acids (FFAs).⁴² Simple hydrolysis of PS can also occur at the FA ester bond, leading to the formation of FFA and polyoxyethylene-sorbitans. This hydrolysis reaction occurs at a slow to negligible rate in most mAb products under normal storage conditions at 2°C–8°C.³⁸ However, recent publications have reported on hydrolysis of PS in products caused by lipases and esterases arising from host-cell proteins that are copurified with the desired protein product. This may become especially apparent

in high protein concentration products.^{43,44} Degradation and thus the loss of functional PS and the presence of FFAs increase the risk of formation of protein aggregates, which has the potential to enhance product-related immunogenicity.^{42,45} More critically, species containing peroxides and free radicals formed from polysorbate degradation can lead to oxidative modification of the mAb at susceptible sites.^{46,47} Oxidative modification of proteins can enhance their immunogenic properties.^{48,49} Similarly, oxidative degradation products of polysorbates such as the aldehydes could also react with the mAb to create reactive carbonyl adducts that may have significant immunomodulatory properties, leading to the generation of antibodies against the mAb.^{50–52} Since the degradation of both PS80 and PS20 follow similar mechanisms notwithstanding the differences in their composition, the aforementioned risks are applicable to products containing either of the polysorbates.

Polysorbates are not physiologically inert. Systemic administration of PS80 at high doses leads to transient hypotension (human dosed 300 mg in 10 min; ≥ 5 mg/kg dosed to dogs) and tachycardia.^{53–55} Weiszhar et al.⁵⁶ have shown that PS80, and to a lesser extent PS20, can directly activate complement at micellar concentrations. Both surfactants lead to a significant production of pleiotropic proinflammatory mediator, C3a, in human blood. PS80 was more effective than PS20 in activation of the terminal complement pathway leading to activation of C5 convertases and production of C5a, an anaphylotoxin. They hypothesized that acute hypersensitivity and systemic immunostimulation during IV administration of products containing these excipients is a result of the complement activation–derived inflammatory mediators.⁵⁶

The medical literature is replete with examples of clinical adverse effects from the parenteral administration of polysorbates to humans. Systemic administration of IV Docefrez[®] and Jevtana[®] (each containing very high concentrations of PS80 compared with mAbs) has been reported to lead to hypersensitivity reactions in 5%–40% of treated patients despite pretreatment with antihistamines and corticosteroids.^{57,58} Coors et al.⁵⁹ identified PS80 as a potentiator of nonimmunologic anaphylactoid reaction after IV administration of a multivitamin product containing PS80. (It must, however, be noted that in the case of small molecule drugs, surfactants are generally added as solubilizers, and thus used in significantly larger amounts compared with biologics). Steele et al.^{60,61} reported on 2 patients who developed hypersensitivity reactions to darbepoetin and erythropoietin. Subsequent skin testing suggested that the reactions were due to PS80 in the products. When these patients were treated with a polysorbate-free product, they had no adverse reactions. Badui et al.⁶² reported generalized urticaria, angioedema, rhinoconjunctivitis, dyspnea, and wheezing, 1 h after the third IM administration of quadrivalent human papilloma virus vaccine. Skin testing performed to PS80 was positive.

The history of reactions to Xolair[®], which contains PS20 as an excipient, is intriguing.⁶³ After all, how can a drug, which by design lowers serum IgE levels and reduces allergic symptoms, be associated with an increased risk of anaphylaxis⁶⁴? Although it contains some murine amino acid sequences, HADA was detected in only 1 of 1723 patients receiving Xolair[®] in clinical trials. Yet initial post-marketing data reported anaphylaxis occurring in 124 Xolair[®]-treated patients, with 39% of these cases occurring to the first dose of Xolair[®]. The frequency of anaphylaxis attributed to Xolair[®] use was estimated to be at least 0.2% which is greater than the incidence of the development of HADA 1/1723 (<0.1%). ISRs are also reported in clinical trials in 45% of Xolair[®]-treated patients and in 43% of treated patients receiving the excipient as a placebo.⁶⁵ The excipients in the formulation are PS20, L-histidine, and sucrose. Dreyfus and Randolph⁶⁶ reported a case of an anaphylactoid and

serum sickness reaction to Xolair[®]. Price and Hamilton⁶⁷ proposed that anaphylaxis seen in 1 of his 2 patients (after more than a year of treatment; positive intradermal test to polysorbate) could have been driven by an IgG macrophage-mediated event because there was no detectable tryptase. Price surmised from the Dreyfus report of serum sickness reaction to Xolair[®] that an IgG-mediated reaction existed as well.⁶⁷ Other causes for anaphylaxis besides polysorbate hypersensitivity have also been proposed. Hypothesis for anaphylaxis include the presence of preexisting antiallotypic or anti-idiotypic IgE or IgG antibody against omalizumab or concomitant causes unrelated to Xolair[®].⁶⁸ A recent report of anaphylaxis in a patient on first administration of Xolair[®] also reported a positive skin prick test (SPT) to polysorbate 20. Interestingly, the patient showed positive SPT to 5 other antibodies containing polysorbates and one conjugated to polyethylene glycol (PEG). A subsequent SPT to PEG also was positive.⁶⁹

Finally, polysorbates are known to be able to elicit type IV immunological reactivity seen commonly as contact dermatitis.⁷⁰ Free radical species and other oxidation products of polysorbates such as aldehydes and ketones function as haptens, likely reacting with proteins at the exposure site to form neoantigens that are recognized by the body as being foreign, as illustrated in Figure 3. Specific memory T-cells are produced which on subsequent exposure to the low-molecular-weight compound results in activation of the immune system. Degraded polysorbate is therefore a contact allergen.^{74,75} As noted earlier, oxidative- and aldehyde-modified proteins are known to be potent inducers of the adaptive immune response.^{48–52} Free oleic acid, present as residue from manufacturing or formed by degradation during storage, has also been proposed as a cause of hypersensitivity reactions in docetaxel and other therapies which use PS80,⁷⁷ while other reports also propose an adjuvant-like activity in modified FAs.^{63,78,79} Mueller et al.⁸⁰ reported that degradation products of polysorbate, possibly related to oleic acid component, upregulated the expression of CD86 costimulatory cytokines by dendritic cells.

Putting the various reports together suggests that in many of the reported cases of clinical adverse effects attributed to the co-administered excipient polysorbate, it may actually be the

degradation of polysorbate that may be the causative factor, and the event may be IgG mediated. The reports in the medical literature do not (and have no possible way to) address the quality of polysorbate in the implicated product. (Along the same lines, the quality of the polysorbate used in the intradermal test in this case report is unknown, and thus the exact cause of the reaction cannot be deconvoluted.) On the other hand, the biochemical studies on degradation of polysorbate do not make the connection from the risk for protein modification to its immunological implication. The amount of protein modification (as adduct instead of the usually studied oxidized form) required for triggering immunogenicity may be quite small and analytically hard to detect. However, because T-cell-mediated and B-cell-mediated responses have been well documented for such oxidative- and aldehyde-modified neoantigens or autoantigens, the connection proposed previously is reasonably likely. Susceptibility may arise from prior or repeated exposure, and in certain individuals may not require a large amount of the antigen to create such a response. There are also numerous reports in the literature of anti-PEG (polyethylene glycol $\equiv$ polyoxyethylene) antibodies present in the population due to general exposure to PEGs via consumer products.⁸¹ It has been observed that anti-PEG antibodies also bind polysorbates (Prof. Carpenter, unpublished personal communication). Because polysorbates also contain the polyoxyethylene motif, the ability to cross-react may not be surprising, but it also points to the possibility of sensitization, not just directly via exposure to polysorbates but also through other moieties such as PEG. The ISRs could thus be a response to pre-existing anti-PEG antibodies and would also explain the positive SPT to PEG in the study by Perino et al.⁶⁹

The higher incidence of ISRs in patients receiving Praluent[®] (vs. placebo) in ODYSSEY outcomes suggests that alirocumab adds to the background immunogenicity seen from polysorbate 20.⁸² While this was not the case with Repatha[®], similar observations of higher ISRs in active versus placebo are not uncommon. Note that in all these cases, the placebo contains the full set of excipients including the polysorbate. (If the control arm of the clinical trial used polysorbate-free placebo (or simply normal saline), it would be expected that the rate of ISRs would be near zero for similar dose volumes and injection frequencies^{83,84}.) Because ISRs are seen in the placebo-treated patients also, we hypothesize that polysorbate (degradation)-induced haptenization is the likely cause. If the ISR rate is higher (than placebo) in the active treated patients, the additional contribution is potentially due to the formation of modified mAb (with polysorbate degradant as hapten adduct) neoantigens.

Conclusions

This report of PCSK9i monoclonal antibody excipient-related polysorbate hypersensitivity adds to the body of literature on this topic. Polysorbate-induced hypersensitivity may be a significant factor in many of the ISRs and even in some of the SAEs to other fully human monoclonal antibody therapies and other biologics.

Sensitization to polysorbate may occur during therapy or it may preexist in susceptible individuals because these (and other nonionic surfactants) are widely used, including in cosmetic and food products. Developing polysorbate hypersensitivity may place these individuals at risk for potentially more serious adverse reactions when other medications containing polysorbates are administered. Developing polysorbate hypersensitivity also reduces the ability of these individuals to benefit from the use of a broad range of therapeutics (as seen by Perino et al.⁶⁹).

Further research is needed to understand the detailed mechanism for hypersensitivity and ISRs, due to the ubiquity of polysorbates in biologics including vaccines. Is the causative factor the polysorbate itself or the degradation product(s), as hypothesized

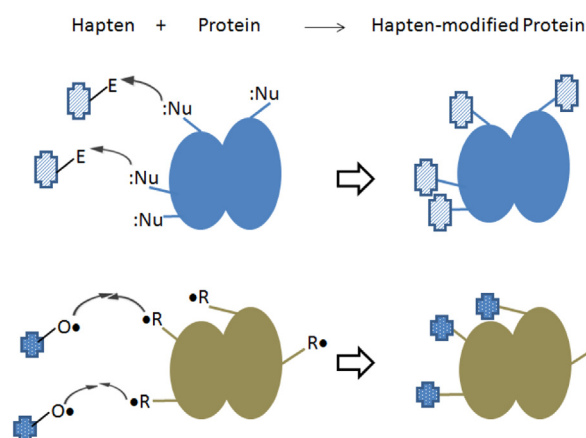


Figure 3. Schematic illustration of hapten–protein reactions (modified from Bergh⁷¹ with permission). Haptens are small molecules with electrophilic or free-radical-forming structural features. Electrophiles target the amino acids with nucleophilic side chains, especially lysine ϵ -amino group, cysteine sulfhydryl group, histidine imidazole group, and the N-terminal amino group.⁷² Species with radicals, containing an uneven number of electrons, are highly reactive and can modify several groups on the protein (with Met, Cys, Tyr, His, Trp being the most susceptible).⁷³ Haptens generated from polysorbate degradation can react with proteins of the immune system such as major histocompatibility complex on antigen-presenting cells such as the Langerhans cells, thus activating these cells.^{71,74–76} Such haptens could also readily modify the active protein itself.

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