



Immunogenicity of Botulinum Toxin Formulations: Potential Therapeutic Implications

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ABSTRACT

Botulinum neurotoxins (BoNTs) are proteins produced by bacteria of the *Clostridium* family. Upon oral ingestion, BoNT causes the neuroparalytic syndrome botulism. There are seven serotypes of BoNT (serotypes A-G); BoNT-A and BoNT-B are the botulinum toxin serotypes utilized for therapeutic applications. Treatment with BoNT injections is used to manage chronic medical conditions across multiple indications. As with other biologic drugs, immunogenicity after long-term treatment with BoNT formulations may occur, and repeated use can elicit antibody formation leading to clinical nonresponsiveness. Thus, approaching BoNT treatment of chronic conditions with therapeutic formulations that minimize stimulating the host immune response while balancing patient responsiveness to therapy is ideal. Immunogenicity is a clinical limitation in many settings

that use biologic drugs for treatment, and clinically relevant immunogenicity reduction has been achieved through engineering smaller protein constructs and reducing unnecessary formulation components. A similar approach has influenced the evolution of BoNT formulations. Three BoNT-A products and one BoNT-B product have been approved by the Food and Drug Administration (FDA) for therapeutic use: onabotulinumtoxinA, abobotulinumtoxinA, incobotulinumtoxinA, and rimabotulinumtoxinB; a fourth BoNT-A product, daxibotulinumtoxinA, is currently under regulatory review. Additionally, prabotulinumtoxinA is a BoNT-A product that has been approved for aesthetic indications but not therapeutic use. Here, we discuss the preclinical and clinical immunogenicity data that exist within the scientific literature and provide a perspective for considering immunogenicity as a key factor in choice of BoNT formulation.

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Key Summary Points

Immunogenicity is a frequent clinical barrier seen with the use of many biologic drugs, including botulinum toxins utilized for therapeutic applications; repeated use can lead to the development of neutralizing antibodies (nAbs) that may affect treatment outcomes

Common strategies for reducing the immunogenicity of biologic drugs and the prevalence of neutralizing antibodies include engineering smaller proteins and reducing contaminants or unnecessary formulation components

For botulinum neurotoxin (BoNT) formulations utilized for therapeutic applications, incobotulinumtoxinA is the most purified; preclinical and clinical data suggest it has reduced immunogenicity compared with other formulations

BoNT therapy is often lifelong in patients with chronic conditions; thus, the potential for immunogenicity and risk of reducing nAb production should be considered when making treatment decisions regarding BoNT formulation

INTRODUCTION

Botulinum neurotoxin (BoNT) injections are used to manage chronic medical conditions across multiple indications and aesthetic applications, including symptomatic relief of blepharospasm, cervical dystonia, various types of focal muscle spasticity, and temporary improvement of dynamic facial lines [1–3]. The therapeutic use of BoNT in chronic conditions is potentially lifelong, and, given the bacterial origins of BoNT, repeated exposure can elicit antibody formation leading to clinical nonresponsiveness [2, 3]. Reports of immunogenicity after long-term treatment with some BoNT

formulations are increasingly emergent [4–13], with varying outcomes dependent on factors such as diagnosis, BoNT formulation, prior BoNT treatment, neurotoxin complex protein load, injection session dose, treatment duration, and length of reinjection interval [3, 14–16].

Multiple BoNTs are approved for use in numerous countries worldwide; first-generation BoNT formulations contain a core neurotoxin plus complexing accessory clostridial proteins, whereas second-generation BoNTs lack complexing, accessory clostridial proteins as a result of their removal during purification [17–21]. These BoNT formulations are associated with varying incidence rates for development of neutralizing antibodies (nAbs) that bind to BoNT and may reduce efficacy or duration of clinical response [17–20]. Additionally, the US Food and Drug Administration (FDA) has issued guidance documents on assessing immunogenicity of therapeutic protein products and recommends a “risk-based approach to evaluating and mitigating immune responses to, or adverse immunologically related responses associated with, therapeutic protein products” [22, 23]. This emerging body of evidence [17–20] and current guidelines [22, 23] emphasize the importance of approaching BoNT treatment of chronic conditions with protocols to minimize the immune response and maximize patient responsiveness.

Immunogenicity of biologic drugs is a clinical limitation in many settings, and observing how other therapies have evolved could provide insights for reducing immunogenicity in BoNT treatment paradigms [24–26]. This article reviews the existing literature and does not contain any new studies with human participants or animals performed by any of the authors. In this review, we describe the basic science of immunogenicity as a potential clinical barrier to the efficacy of biologic therapies and its effect on the evolution of BoNT formulations. We summarize available nonclinical and clinical evidence of immunogenicity and clinical nonresponsiveness associated with different BoNT formulations and discuss whether there is a lower risk of immunogenicity with a second-generation BoNT formulation,

incobotulinumtoxinA. Finally, we discuss potential areas of research to address current knowledge gaps and provide an immunologic perspective for considering immunogenicity as a factor in choosing a BoNT formulation.

IMMUNOGENICITY AND CLINICAL LIMITATIONS OF BIOLOGIC DRUGS

Immunogenicity is the ability of any molecule, including foreign proteins or biologic drugs, to provoke a host immune response [27]. Any biologic drug, such as a recombinant therapeutic protein, gene therapy vector, or vaccine, has the potential to become a target of the immune system, particularly if administered repeatedly or at a high dose [27, 28]. For example, protein-based vaccines are designed to trigger adaptive immunity and the development of specific antibodies to potential pathogens to exert their effect [29]. However, immunogenicity is undesirable when the production of antidrug antibodies or other immune processes lead to a loss of therapeutic effectiveness of a biologic drug, which can occur through direct neutralization as well as alteration in pharmacokinetics [27].

Immunogenicity in Response to Biologic Drugs

The recognition of biologics and induction of an immune response are mediated by a network of immune cells, including antigen presenting cells (APCs), T cells, and B cells (Fig. 1) [30]. Dendritic cells are a type of APC that engulfs foreign proteins, such as biologics, by capturing them through various cell-surface receptors, including Toll-like receptors (TLRs), Fc receptors, and members of the C-type lectin family [31]. Dendritic cells can then process the captured biologic and display peptide fragments on the cell surface via the major histocompatibility complex (MHC) [30, 31]. Peptide-MHC complexes are presented to and recognized by T cells, which in turn can stimulate B cells to produce antigen-specific antibodies [30, 31]. The activation of T cells is also dependent on the presence and binding of costimulatory

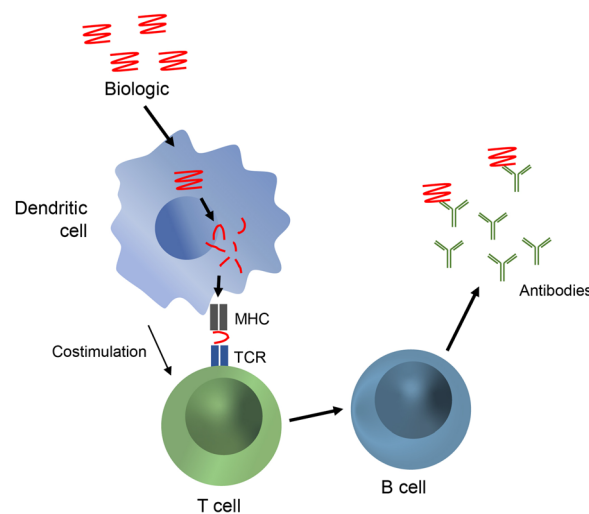


Fig. 1 Immunogenicity in response to biologic drugs. Dendritic cells engulf biologics that bind to various cell surface receptors [27, 31]. Biologics are processed and presented as peptide fragments on the cell surface via the major histocompatibility complex (MHC) [30, 31]. Peptide-MHC complexes are presented to and recognized by T cells, which stimulate B cells to clonally expand and produce antigen-specific antibodies [30, 31]. Costimulation by molecules such as cytokines is required for complete activation of T cells. Subsequent expansion of B cells results in long-lasting and abundant production of antibodies [30, 31]. *TCR*, T-cell receptor

molecules, including cytokines, that are produced when the APCs are activated by stimulation of surface receptors (e.g., TLRs). Subsequent activation and expansion of B cells can result in long-lasting and abundant production of antibodies through memory B cells or plasma cells, a hallmark of adaptive immunity [30, 31]. Additionally, antibodies against biologics can be generated through T-cell independent pathways, where aggregates of the biologic can directly bind and stimulate B cells to produce antibodies [31, 32]. Some of the antibodies produced through either T cell-dependent or T cell-independent pathways are nAbs that can inhibit the activity and nullify the therapeutic effect of the biologic [27].

The immunogenicity can be influenced by several key factors, including molecular weight, structural complexity, posttranslational modifications, and features of the amino acid sequence [27, 33]. However, smaller molecules

that may not be immunogenic alone can bind to larger endogenous proteins and be recognized by the immune system, leading to activation of dendritic cells and an adaptive immune response [34]. Biologics that form large aggregates may also be able to interact with and activate B cells [31, 35]. Additionally, other components of the biologic formulation, such as excipients (e.g., surfactants) and contaminants (e.g., host cell proteins, including bacterial flagellin) present in injected treatments, may stimulate the immune system and result in an increase in neutralizing antibodies against a biologic due to an unintended adjuvant effect [26, 36]. This adjuvant effect has been suggested to initiate an innate immune response after exposure to flagellin via TLR5 [9, 37].

Following the initial priming of the adaptive immune response, repeated exposure to the antigen may result in a faster and stronger response [30, 38]. In the case of biologic drug therapies, including BoNT therapy, repeated clinical exposure can potentially provoke a continual immune response and formation of nAbs, resulting in a spectrum of clinical non-responsiveness outcomes [31, 39–42]. Common signs of clinical resistance include increasing the frequency or dose of the drug administered to elicit results, complete nonresponse, and partial nonresponse (Fig. 2) [25, 42, 43].

Clinical Challenges of Immunogenicity to Biologic Drugs

Biologic drugs have revolutionized treatment of many conditions across all areas of medicine [25]. Nevertheless, immunogenicity is a key clinical challenge associated with the use of many biologic therapies [30]. The formation of nAbs and antibodies against formulation contaminants has been observed in several therapeutic areas, including Fabry disease [24], rheumatoid arthritis [25], insulin-dependent diabetes [44], and asthma [26], among others (also seen in inflammatory bowel disease, psoriasis, and psoriatic arthritis). In Fabry disease, a rare X-linked disorder, enzyme replacement therapy (ERT) can lead to dose-related development of nAbs, which in turn limits treatment efficacy and results in disease progression, loss of renal function, and adverse cardiovascular outcomes [24, 45, 46]. However, data from patients with Fabry disease and renal transplants have shown that administration of immunosuppressant drugs before ERT prevents the formation of nAbs, suggesting that immunomodulation prior to ERT in patients at risk for clinically significant antibody development may be a promising approach to nAb management [24, 47].

The formation of nAbs is a common concern in patients with rheumatoid arthritis receiving biologic tumor necrosis factor α (TNF- α) inhibitors, and secondary loss of response may require switching to a treatment with an

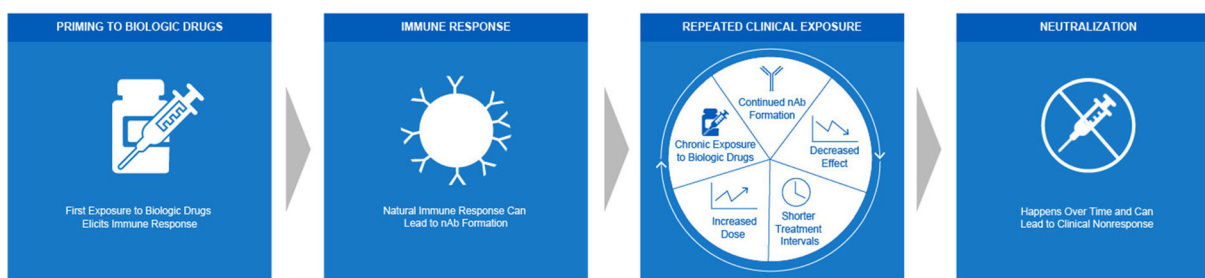


Fig. 2 Immunogenicity drives clinical nonresponsiveness. Repeated clinical exposure to biologic drugs can provoke a continual immune response and formation of nAbs leading to clinical resistance [30, 31, 39]. Common signs of clinical

resistance include increasing the frequency or dose of the drug administered to elicit results. Over time, this can lead to clinical nonresponse [25, 42, 43]. *nAb*, neutralizing antibody

alternative mechanism of action [25, 48]. Approximately 30% to 40% of patients discontinue use of biologic TNF- α inhibitors because of nonresponse or intolerance [48], and the risk of nAb development can vary across TNF- α inhibitors, in part because of structural differences between antibody constructs. A reduction in immunogenicity has been achieved by developing smaller, more targeted fusion proteins rather than using large chimeric or fully humanized antibodies [25, 49, 50].

Similarly, treatment for diabetes has evolved over time to address immunogenicity issues in insulin formulations related to host-derived (e.g., bovine, porcine, human) structural differences, insulin purity, formulation additives (e.g., zinc, protamine, surfen), and drug aggregation [44]. Treatment with initial insulin formulations showed both insulin-specific nAbs and antibodies to other drug components [44]. However, replacing impure animal insulins with highly purified porcine insulins and, more recently, recombinant and semisynthetic human insulin preparations has vastly reduced—although not completely eliminated—the occurrence of immunogenicity [44, 51, 52].

As seen with early insulin formulations, immunogenicity to formulation additives and contaminants presents additional clinical challenges that have emerged in other therapeutic applications of biologic drugs [26]. Initial trials of lebrikizumab, an investigational treatment for asthma, were conducted with a formulation that contained a Chinese hamster ovary (CHO) cell protein contaminant, which provoked a measurable immune response in ~ 90% of patients. As a result, the ongoing phase 3 studies were converted to phase 2b and were no longer considered pivotal. Further purification of material was required, and drug manufacturing protocols were adjusted to reduce the CHO contamination, which led to a reduction in immunogenicity in subsequent trials [26].

Immunogenicity is a common clinical barrier to therapy with many biologic drugs, and it has influenced the evolution of biologic treatments across multiple disease states. Some common strategies to reduce general immunogenicity and the prevalence of nAbs include

engineering smaller proteins and reducing contaminants or unnecessary formulation components [31]. These general themes can inform the understanding of immunogenicity of BoNT therapy.

IMMUNOGENICITY AND EVOLUTION OF BOTULINUM NEUROTOXIN BIOLOGIC THERAPY

Botulinum Neurotoxin Structure and Function

BoNTs are proteins produced by bacteria of the *Clostridium* family, which upon oral ingestion cause the neuromuscular syndrome botulism. There are seven serotypes of BoNT (serotypes A–G) with different toxicities but similar structures. In all serotypes, the bacterial complex comprises a core ~ 150-kDa neurotoxin surrounded by a group of associated accessory proteins (Fig. 3A, B) [53, 54]. These accessory proteins assemble into a supramolecular structure that supports the dual function of protecting the core neurotoxin from low pH conditions upon oral ingestion and facilitating gastrointestinal absorption [54].

At low pH, the core neurotoxin is surrounded by an assembly of one nontoxic non-hemagglutinin (NTNH) protein plus a complex of hemagglutinin (HA) proteins [54, 55]. The ~ 140 kDa NTNH protein plays a key role in protecting BoNT from protease digestion and low pH degradation in the stomach. However, at a neutral pH in the small intestine, the “pH-sensor” residues of the NTNH protein induce a conformational change releasing the core neurotoxin [56]. The HA proteins (HA1, HA2, and HA3) mediate cell-surface binding and translocation across the intestinal epithelium. The NTNH protein, core neurotoxin, and HA complex assemble to form the final supramolecular structure [55, 57].

The core neurotoxin itself is formed of a heavy (100 kDa) chain and a light (50 kDa) chain linked by a disulfide bond [54, 55]. The role of the heavy chain is to bind to presynaptic cholinergic terminals in the neuromuscular

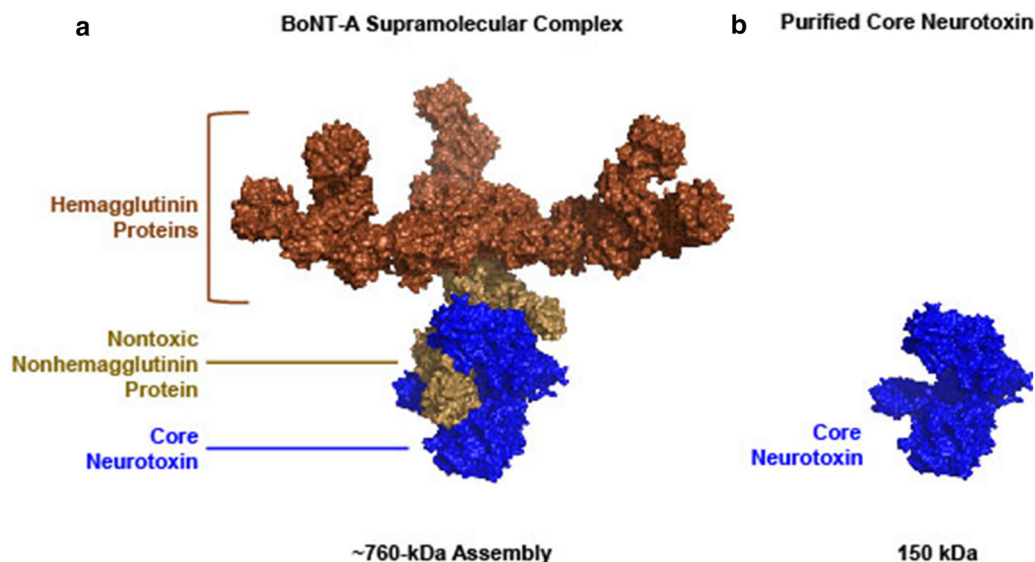


Fig. 3 Molecular structures of botulinum neurotoxin type A in **A** supramolecular complex and **B** purified core neurotoxin. **A** BoNT-A is a supramolecular complex consisting of hemagglutinin proteins, a nontoxic non-hemagglutinin protein, and the core neurotoxin [54, 64], with a molecular weight of up to ~ 900 kDa as produced by the *Clostridium* bacteria [57]. The structural model shown here (representing ~ 760 kDa) was determined using recombinant protein and cryogenic electron

junction to gain cell entry and mediate translocation of the dissociated light chain to the cell cytoplasm. The light chain is a zinc metalloprotease that cleaves specific target soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins to prevent vesicle fusion and subsequent release of acetylcholine (ACh) causing flaccid paralysis [55]. The seven BoNT serotypes cleave different components of the SNARE complex to achieve this effect. BoNT-A and BoNT-E cleave synaptosomal-associated protein 25 (SNAP-25); BoNT-B, BoNT-D, BoNT-F, and BoNT-G cleave synaptobrevin; and BoNT-C cleaves both SNAP-25 and syntaxin [58]. Blocking ACh release is the mechanism of action of therapeutic BoNT formulations, and BoNT-A and BoNT-B are the botulinum toxin serotypes currently in clinical use [17–20].

microscopy [57] and is currently the largest assembly for which three-dimensional structural information is available. **B** Purified BoNT-A formulations contain only the core neurotoxin (~ 150 kDa) [54, 64]. Images rendered with PyMol (Schrödinger, Inc.) using atomic coordinates from the Protein Data Bank with the following accession codes: 3WIN, 3V0A, 3V0B, 3V0C, and 4LO7 [56, 57, 120]. *BoNT-A*, botulinum neurotoxin type A

Immunogenicity and Evolution of BoNT Formulations: Nonclinical Data

As is possible for other biologic drugs, both the BoNT core neurotoxin and the associated accessory proteins have the potential to be immunogenic [35]. Detoxified BoNT extracts and catalytically inactive BoNT proteins have been used for many years to develop vaccines against botulism [59, 60]. Administration of these vaccines results in production of antibodies that can neutralize the toxic effects of BoNT [59–62]. For example, antibodies targeting the core neurotoxin that successfully inhibit its neuronal binding or uptake or its catalytic activity have the potential to be neutralizing [35, 59].

Immunogenicity of Accessory Proteins

The accessory proteins in BoNT formulations have a nontherapeutic role and rapidly

Table 1 Characteristics of current first- and second-generation BoNT-A preparations [64, 74, 75, 79, 94, 116–118]

Parameter	First-generation BoNT-A ^a		Second-generation BoNT-A ^b	
	OnabotulinumtoxinA	AbobotulinumtoxinA	IncobotulinumtoxinA	DaxibotulinumtoxinA ^c
Molecular weight of bacterial protein, kDa	~ 900	~ 300–500 ^d	~ 150	~ 150
Accessory proteins present	Yes	Yes	No	No
Total protein/vial	5 ng/100 U	4.87 ng/500 U	0.44 ng/100 U	NA
Total core neurotoxin protein/100 MU, ng	0.73	0.65	0.44	NA
Active neurotoxin protein/100 MU, ng	0.44	0.44	0.44	NA
Inactive neurotoxin protein/100 MU, ng ^e	0.29	0.21	0	NA
pH after reconstitution	7.4	7.4	7.4	NA
Excipients	HSA	HSA	HSA	RTP004 peptide
	NaCl	Lactose	Sucrose	Polysorbate-20
				Buffers
				Sugar

BoNT, botulinum neurotoxin; *FDA*, Food and Drug Administration; *HSA*, human serum albumin; *MU*, mouse unit; *NA*, not available; *NaCl*, sodium chloride

^a First-generation BoNT-A formulations contain core neurotoxins and accessory botulinum proteins; only formulations approved or under investigation for therapeutic applications are represented

^b Second-generation BoNT-A formulations contain only the therapeutic neurotoxin without accessory proteins or other bacterial substances such as flagellin

^c Currently undergoing FDA review; full details on the formulation are not yet available

^d Formulation is a mixture of species, with 300 and 500 kDa being the most common

^e Values for inactive neurotoxin are approximate and were estimated in Frevert et al. 2010 and then reported in Kerscher et al. 2019 [64, 74]

dissociate from the core neurotoxin at neutral pH [54, 63, 64]. Thus, the total clostridial protein load (inclusive of accessory proteins and the core neurotoxin) and composition may determine the relative immunogenicity of each BoNT formulation [1].

There is compelling evidence that accessory clostridial proteins, particularly HA-1, act as adjuvants to the immune response [65–71] and that this activation of the immune system can facilitate the development of therapeutically relevant nAbs against the BoNT core neurotoxin [1, 2]. In mice, injection with the core neurotoxin of the BoNT complex alone has low immunogenicity. In contrast, when HA proteins (especially HA-1 and HA-3b) are injected also, antibody production is significantly increased [69]. Additionally, immunization of rabbits with the full inactivated BoNT complex results in production of antibodies with a greater neutralizing effect compared with antibodies induced by immunization with the core neurotoxin alone [66]. These data support an adjuvant effect of accessory proteins as injections were administered in a neutral pH buffer in which the BoNT complex would be dissociated [66, 69].

In addition, accessory proteins may induce other immune-mediated effects. In vitro assays show that in the presence of BoNT accessory proteins, neuronal cells increase production of inflammatory cytokines such as IL-6 and TNF- α [71]. Accessory proteins also bind to multiple nonneuronal cell types, including fibroblasts, lymphoblasts, and skeletal muscle cells [71]. In contrast, the core neurotoxin does not bind to nonneuronal cell types and does not induce cytokine release [71].

On the basis of available preclinical data, a BoNT formulation containing as little clostridial protein as possible is desirable as it may avoid stimulating the host immune response leading to nAb formation and clinical nonresponse [1]. Three BoNT-A products are approved by the FDA for therapeutic use: onabotulinumtoxinA (onaBoNT-A; Botox®; Allergan Pharmaceuticals), abobotulinumtoxinA (aboBoNT-A; Dysport®; Ipsen Biopharm Ltd; Galderma Ltd), and incobotulinumtoxinA (incoBoNT-A; Xeomin®; Merz Pharmaceuticals GmbH) [17–19]; a fourth

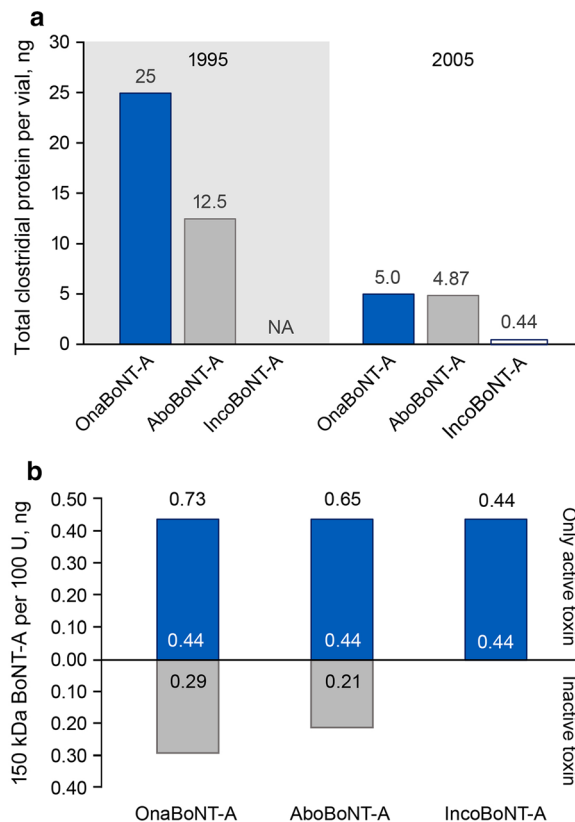


Fig. 4 Total clostridial protein and active core neurotoxin in BoNT-A formulations. **A** Changes in total clostridial protein content in onaBoNT-A [1, 121], aboBoNT-A [1, 74], and incoBoNT-A [1] formulations over time. Current formulations are presented in the 2005 panel. **B** Total core BoNT-A neurotoxin in onaBoNT-A, aboBoNT-A, and incoBoNT-A formulations separated into denatured/inactive toxin and active neurotoxin components [64, 74, 75]. *aboBoNT-A*, abobotulinumtoxinA; *BoNT-A*, botulinum neurotoxin type A; *incoBoNT-A*, incobotulinumtoxinA; *NA*, not available; *onaBoNT-A*, onabotulinumtoxinA

BoNT-A product, daxibotulinumtoxinA (daxiBoNT-A; Revance Therapeutics), is currently under regulatory review for a nontherapeutic indication [72]. These products vary in the amount of accessory proteins and the excipients (e.g., albumin; Table 1) [17–19]. PrabotulinumtoxinA is a BoNT-A product that has only been investigated and approved for use in the treatment of glabellar facial lines and is not included in our discussion of therapeutic applications [21, 73]. Additionally, one BoNT-B formulation, rimabotulinumtoxinB (rimaBoNT-B; Myobloc®;

Table 2 Incidence rates of development of nAbs and clinical nonresponsiveness with current BoNT formulations

	First generation			Second generation
	BoNT-A ^a		BoNT-B	BoNT-A
	OnabotulinumtoxinA	AbobotulinumtoxinA	RimabotulinumtoxinB	IncobotulinumtoxinA
Patients with nAbs in pivotal clinical trials	0.0%–1.9% [17]	0.0–3.6% [19]	10–18% [20]	0–1.8% [18]
Patients with nAbs in real-world studies [3, 5]	1.5–7.0%	1.7–6.0%	42.4%	0.0–0.5%
Reports of clinical resistance/nonresponse	Yes	Yes [90–92]	Yes	No
Formulation notes	Reduced protein load from original formulation [119] (i.e., reduced clostridial protein impurities and inactive BoNT-A) [74]	Contains flagellin with potential adjuvant properties [9, 37]; contains complexing proteins [74]	Contains complexing proteins	No complexing proteins; no inactive toxoids [74]; no patients with secondary nonresponse

BoNT-A, botulinum neurotoxin type A; *BoNT-B*, BoNT type B; *nAb*, neutralizing antibody

^a Antibody assays used to detect nAbs for first-generation onabotulinumtoxinA and abobotulinumtoxinA formulations were less sensitive than that used for the second-generation incobotulinumtoxinA formulation [2, 43]

Solstice Neurosciences, LLC), is FDA approved and contains the core neurotoxin and the accessory proteins [20]. The different protein loads, excipients, and other characteristics of these BoNT formulations may affect their immunogenicity potential [9, 43].

BoNT-A Formulations with Accessory Proteins

OnabotulinumtoxinA (onaBoNT-A) was the first BoNT-A formulation to be approved by the FDA in 1989 [17]. The initial formulation contained a large proportion of inactivated core neurotoxin plus clostridial proteins, and up to 17% of patients developed anti-BoNT antibodies [2]. In

1997, a less immunogenic formulation containing a reduced quantity of inactivated neurotoxin was manufactured (Fig. 4A) [2]. Currently, onaBoNT-A contains 0.73 ng of core neurotoxin protein (a mixture of active protein and inactive/denatured toxoid) and ~ 4.3 ng of additional accessory protein (Fig. 4, Table 1) [1, 10, 74, 75].

In vivo evidence indicates that anti-BoNT antibodies are produced in response to onaBoNT-A injections, and more frequent dosing is associated with higher antibody levels [76]. Furthermore, in rabbits given nine doses of onaBoNT-A or incoBoNT-A (at 2- to 8-week intervals), nAbs were detected in 20% of

onaBoNT-A-treated animals. In contrast, no nAbs were detected in animals treated with the incoBoNT-A formulation containing only the core neurotoxin, suggesting an adjuvant effect of the accessory proteins and high core neurotoxin concentration in onaBoNT-A [77].

The other first-generation BoNT-A formulation abobotulinumtoxinA (aboBoNT-A) contains less clostridial protein than the current formulation of onaBoNT-A; however, accessory proteins make up ~ 30% of the total clostridial protein content (Table 1; Fig. 4) [1, 74]. The aboBoNT-A formulation also contains the non-BoNT clostridial flagella remnant (flagellin), which has been shown to activate TLR5, initiating an innate immune response [9, 37]. Given its immune stimulating properties, flagellin has been utilized as a vaccine adjuvant and may contribute to the immunogenicity of aboBoNT-A [9, 37, 78].

BoNT-A Formulations Without Accessory Proteins

Second-generation BoNT-A formulations (incoBoNT-A and the investigational product daxiBoNT-A) contain only the therapeutic neurotoxin without accessory proteins or other bacterial substances such as flagellin (Table 1, Fig. 4) [1, 18, 64, 74, 75, 79]. The absence of accessory proteins in incoBoNT-A has no effect on efficacy [1, 80]. The formulation of daxiBoNT-A is unusual in that it does not contain human serum albumin (HSA) and instead contains a proprietary, HIV-derived 5-kDa stabilizing peptide and polysorbate 20 [79, 81]. The adjuvant effects of these excipients—particularly the novel HIV-derived peptide that has not been used in any other drug formulation approved by the FDA—are not yet known.

Immunogenicity and Clinical Nonresponsiveness During BoNT Treatment: Clinical Data

BoNT treatment can cause an adaptive immune response with repeated injections leading to nAb formation over time [7, 10]. This is clinically relevant because nAb formation is strongly associated with secondary nonresponse

(treatment resistance) [82–86]. There are multiple clinical risk factors for immunogenicity during BoNT treatment related to treatment parameters, patient characteristics, and choice of BoNT formulation [2, 3, 14].

BoNT Formulations: Immunogenicity Data

Development of nAbs is possible with all formulations of BoNT; however, the rate of nAb development and occurrence of clinical resistance appear to be at least partially dependent on the BoNT formulation and correlated to the protein content in the formulation [43]. Incidence rates vary by indication and, in pivotal trials supporting FDA approvals of BoNT formulations in clinical use, nAbs developed in patients treated with rimaBoNT-B, onaBoNT-A, aboBoNT-A, or incoBoNT-A (in patients all of whom had been pretreated with onaBoNT-A or aboBoNT-A; Table 2) [17–20]. It is also important to note that there are differences in the relative sensitivity of assays used by BoNT manufacturers to detect the presence of nAbs, which can complicate making direct comparisons [2]. Pivotal studies supporting FDA approval of onaBoNT-A, aboBoNT-A, and rimaBoNT-B utilize the mouse protection assay (MPA); in contrast, most studies of incoBoNT-A use the mouse hemidiaphragm assay (MHDA), which is at least five times more sensitive than the MPA and nevertheless revealed the lowest rates of nAb formation (Table 2) [2, 43]. Additionally, the reported incidence rates of nAbs in product labeling are based on data from short-term clinical trials (~ 2 years) and may not reflect real-world data given there may be a cumulative effect of repeated BoNT use over time [43].

However, similar trends are seen in real-world studies with long-term follow-up analysis showing reduced nAb presence in patients treated with incoBoNT-A [5, 82, 87]. A retrospective meta-analysis suggests more prevalent nAbs across indications in patients treated with onaBoNT-A (~ 1.5%) or aboBoNT-A (~ 1.7%) compared with incoBoNT-A (0.5%) [5]. While overall prevalence is low, there was a considerably higher rate of nAb development among patients who were identified as demonstrating secondary nonresponse in this meta-analysis

[5]. Among such patients, nAbs were reported in 32.5% of those treated with onaBoNT-A and 56.7% of those treated with aboBoNT-A [5]. Importantly, no patients treated with incoBoNT-A demonstrated secondary nonresponse [5].

BoNT-A Formulations with Accessory Proteins

Occurrence of nAbs after treatment with onaBoNT-A or aboBoNT-A has been reported in patients with cervical dystonia, spasticity, and other indications [3, 9, 12, 82, 88, 89]. In a cross-sectional study of patients with facial hemispasm, blepharospasm, cervical dystonia, other dystonia, and spasticity, nAbs were reported with use of both onaBoNT-A (7%) and aboBoNT-A (6%) but not incoBoNT-A [3]. These findings are consistent with a recent retrospective cohort study of long-term BoNT treatment across indications, which showed nAb-induced treatment failure in patients who received onaBoNT-A (4%) or aboBoNT-A (16%) but not in those who received incoBoNT-A [82]. Furthermore, there is evidence from case studies of BoNT-A use for aesthetic indications of nAb development and secondary nonresponsiveness over time with both onaBoNT-A and aboBoNT-A [90–92]. In general, incidences of nAb development and secondary nonresponsiveness are lower in aesthetic indications, which may reflect the lower doses and minimal long-term data [9, 92].

BoNT-A Formulations Without Accessory Proteins

The development of antibodies and likelihood of clinical nonresponse are reduced with formulations of BoNT without accessory proteins [43]. No toxin-naïve patients treated with incoBoNT-A developed neutralizing antibodies, based on the sensitive MHDA assay [16]. Furthermore, no reports of clinical nonresponse exist in the medical literature for patients who were toxin-naïve when they received incoBoNT-A [2, 16]. In a recent study examining two patient cohorts, those treated exclusively with incoBoNT-A did not show any signs of secondary treatment failure, whereas those previously treated with other BoNT formulations

were more likely to develop such signs [16]. This study also showed that switching to incoBoNT-A after secondary treatment failure with another BoNT formulation helped patients begin to recover responsiveness to treatment; development of nAbs only occurred in two patients previously treated with aboBoNT-A [16]. Additionally, pooled analysis data from pivotal clinical studies across aesthetic indications reported no lack of treatment response due to development of nAbs [93].

Out of > 2600 patients treated with incoBoNT-A in pivotal clinical trials across all approved indications, nAbs developed in only nine adult (5 with unknown and 4 with negative nAb status at baseline) and four pediatric (nAb status unknown at baseline) patients pretreated with either onaBoNT-A or aboBoNT-A, and none exhibited secondary treatment failure due to nAbs [18]. It is promising that no incoBoNT-A-treated children developed resistance during clinical trials [18], as these patients potentially require lifelong treatment with BoNT starting from an early age and are potentially at greater risk to develop an immune response.

An investigational BoNT-A formulation daxibotulinumtoxinA has been evaluated in phase 3 clinical trials for aesthetic use, but long-term data are not yet available, and immunogenicity outcomes are yet to be established [94].

Treatment Parameters

Multiple treatment parameters affect BoNT immunogenicity. Most importantly, during a potential life-long treatment, prevalence of nAbs increases with chronic BoNT use—cumulative dose, repeated injections, and total treatment duration [2, 6, 7, 13]. Other parameters, such as protein load, injection session dose, and length of reinjection interval, have also been demonstrated to be clinically relevant to BoNT immunogenicity [3, 6, 13, 15].

Patient Characteristics

The rate of nAb development in clinical practice may be higher under certain conditions; several studies have suggested prevalence rates of ~ 15–20% in patients with cervical dystonia

[3, 5, 6] after long-term treatment vs. ~ 1–6% in patients with limb spasticity [5, 7, 89]. The reasons for these differences are not well understood but may be related to variations in underlying pathophysiology and dosing/administration requirements [2]. Conditions that require more frequent administration or higher doses appear to be associated with a greater risk of immunogenicity [3, 6, 13, 89].

Genetic differences in the control of immune responses indicate that patients exhibit variable speed and magnitude of immune reactions and patterns of nAb generation [36, 95–97]. Furthermore, not all nAbs are the same—variations in target binding site and binding affinity result in antibodies generated against BoNT that vary in their neutralizing effects [2, 96]. Thus, there is not an absolute correlation between appearance of nAbs and treatment resistance, and there does not seem to be a particular threshold for nAb titer above which clinical resistance occurs [2]. However, investigations of such a threshold have been limited. Often, secondary treatment failure may be observed after an initial positive response over several treatment cycles [2, 12].

IMMUNOLOGISTS' PERSPECTIVE: IMMUNOGENICITY SHOULD BE A KEY FACTOR IN CHOICE OF BONT THERAPY

BoNT use may have a cumulative immunogenic effect over time in patients with lifelong conditions, such as cervical dystonia [13, 98], as well as in patients who receive multiple BoNT-A treatments for a range of different indications (both aesthetic and therapeutic) during their lifetime. Both the FDA [22] and the European Medicines Agency (EMA) [99] recommend evaluating and mitigating adverse immunologically related responses associated with therapeutic protein products and encourage risk reduction. Therefore, where efficacy and safety are comparable, a BoNT formulation that is potentially less likely to cause immunogenicity should be considered as a first-line therapy [16, 43].

While they are not interchangeable, clinical study data comparing onaBoNT-A and incoBoNT-A demonstrate similar efficacy and safety when used at similar doses across multiple indications [80, 100–103]. Thus, initiating treatment with a second-generation BoNT-A formulation that has lower potential immunogenicity (incoBoNT-A) may reduce the risk of nAb production and future treatment failure [16, 80]. For patients who had begun treatment with a more immunogenic BoNT formulation (onaBoNT-A or aboBoNT-A), switching to a less immunogenic formulation may be an appropriate choice as the process of developing nAbs can begin very early—before clinical signs of resistance are apparent [12, 43, 104–108]. It is particularly important to proactively and systematically recognize signs of clinical resistance, such as increased dosage and shortened injection intervals [12, 42, 43]. In such cases, changing to a less immunogenic formulation is especially warranted and would ideally occur early enough to prove effective in restoring an optimal clinical response [104, 109].

Switching to a BoNT-B formulation is undesirable from an immunologic perspective, given that BoNT-B has a higher immunogenicity than BoNT-A (Table 2) [110, 111]. Additionally, patients who change from BoNT-A to BoNT-B show a reduced response to BoNT-B over time [111, 112], and resistance can develop within a few cycles of treatment [85, 113, 114]. Overall, in the absence of an ability to test nAbs commercially, available evidence regarding differences between BoNT formulations in immunogenic potential and changes in clinical responsiveness over time should be used to inform treatment decisions.

Data Gaps and Unknowns

Despite the evidence that accessory clostridial proteins can act as adjuvants to the immune response [65–71] and in vivo data linking the first-generation BoNT-A formulations to the formation of nAbs [76, 77], unanswered questions remain relating to the immunogenicity of BoNT formulations. Further study is needed to elucidate the different effects of core

neurotoxin alone and accessory proteins on the immune system, including the role of specific cytokines, TLRs, and other innate immune or pattern recognition markers. It is largely unknown whether inactive denatured toxin (such as that used in BoNT vaccines [59, 60] and present in onaBoNT-A and aboBoNT-A) [74] has any effect on nAb production, although it is of clear concern from a therapeutic effect standpoint. However, initial evidence strongly suggests that nAbs are reduced in formulations that lack accessory proteins (incoBoNT-A) [11, 16, 18], and additional larger studies are needed to confirm this correlation.

Perhaps the largest data gap is the lack of long-term data in pediatric patients who often receive lifelong treatment and may be at higher risk of chronic inflammation and other potential complications due to repeated immune system stimulation. Some data suggest that in pediatric patients treated for spasticity with onaBoNT-A or aboBoNT-A, the likelihood of an immune response increased with number of treatments [86]. However, identifying signs of clinical resistance in pediatric patients is complicated by the fact that they are still growing and may have different trajectories of their underlying disease state compared with adult patients; accordingly, use of increased doses of BoNT over time or earlier waning of clinical effect—often signs of clinical resistance in adults—may not be similarly informative in children. Even so, longitudinal real-world studies to determine development of nAbs and to identify practical assessments of clinical nonresponsiveness in pediatric patients would be highly informative for developing effective treatment paradigms in chronic conditions treated with BoNT. This is also true for other patient populations, and real-world studies could help determine if there is a cutoff for nAb titer related to lack of efficacy in patients who receive multiple injections.

It is important to note that nAb testing offers a single snapshot in time of a patient's antibody titer, but titers may exhibit temporal variations between injections. Thus, regular nAb testing would be helpful, although the current practical limitations, such as cost and high volume of patient serum required for testing, remain a

barrier to implementation [2]. Availability of an affordable commercial nAb test would help to address these current challenges. In the meantime, utilizing other clinically useful tools, such as the ninhydrin sweat test, unilateral brow injection test, and extensor digitorum brevis test, to screen for potential nAbs in patients receiving BoNT are sometimes beneficial [2, 107, 115].

LIMITATIONS

All biologic drugs and therapeutic proteins, including BoNT formulations, can be recognized as foreign by the immune system and, therefore, have the potential for immunogenicity. Detecting immunogenicity via nAb formation is assay dependent given the variability in sensitivity and specificity (sensitivity discussed in Clinical Data section). Assessing the incidence of nAb positivity may be influenced by factors such as assay methodology, handling of samples and timing of collection, concurrent use of medications, and underlying disease pathology. Therefore, directly comparing the incidence of nAbs across BoNT formulations may be misleading. Other limitations of the data presented in this review include the lack of a commercially available quantitative assay to measure nAbs and a lack of studies comparing BoNT formulations with a standardized nAb assay.

CONCLUSIONS

Immunogenicity is a common clinical barrier seen with the use of many biologic drugs. Repeated exposure to biologic therapies, including BoNT, can provoke a continual immune response, leading to formation of nAbs that can result in a spectrum of clinical outcomes (e.g., reduced efficacy and/or treatment failure) [31, 39–42]. In some cases, mitigation of general immunogenicity and nAb formation in response to biologic therapies have been achieved through engineering smaller proteins and reducing contaminants or unnecessary formulation components [31]. Specifically,

accessory clostridial proteins in BoNT formulations, particularly HA-1, may act as adjuvants to the immune response [65–71]. Additionally, protein content of BoNT-A formulations influences nAb formation—higher protein content is correlated with increased nAb induction [43].

Compared with onaBoNT-A and aboBoNT-A, the second-generation BoNT formulation incoBoNT-A contains only the therapeutic neurotoxin (150 kDa) and lacks accessory proteins or other potential adjuvants (e.g., flagellin) [1, 9, 37]. Additionally, incoBoNT-A demonstrates strong clinical efficacy and safety, as well as low immunogenicity across a range of indications [16, 80, 100–102, 108]. Given that BoNT therapy is often lifelong, the potential for immunogenicity and risk of reducing nAb production should be considered when making treatment decisions regarding BoNT formulation.

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