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LETTER TO THE EDITOR

Does *Clostridium botulinum* neurotoxin A exhibit molecular mimicry with thyroid autoantigens and cause thyroid complications in predisposed persons?

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In a recent article, Gregoric et al. [1] predicted *in silico* epitope locations in botulinum neurotoxin A (BoNT/A) and derived "epitope" homologies to TSHR, thyroglobulin and thyroperoxidase (Fig. 4). We noted major concerns among which: (a) predicted epitopes disagree with those actually recognized by human Abs [2, 3]. (b) We found no significant homologies in indicated regions. No more than two or three identical residues are contiguous. Only BoNT region 97–103 has a 4-residue identity (YSTD) with TSHR 146–152. It is uncertain that this will cause a cross-reaction because a 4-residue segment is marginal for binding. Furthermore, it is outside an epitope [3]. (c) No testing by synthetic peptides of predicted areas was done. (d) Placebo injections were not tested. (e) Likelihood of cross-reaction through contaminating clostridial flagellins in Dysport [4] were not considered. We found that flagellins and TSHR have a number of 4- and 5-residue sequence identities. (f) To exclude this, patient injection with another BoNT/A product (three are on the market) is necessary.

After we determined expected match frequencies and confidence ranges (based on protein composition, extended-length matches, or matches with one internal mismatch, 2–7 residues in length), we found no support that the observed matches between BoNT and human TSHR, thyroglobulin and/or thyroperoxidase exceed random expectation. Antigen mimicry through continuous segments between BoNT and any of these three proteins is not supported. Without 3D structural comparisons, "discontinuous" epitopes in two proteins cannot be assessed. Therefore, sequence similarities "*in silico*" longer than ten residues with short homologous regions at distal ends of the sequence require antigen testing.

Authors' P value (0.0017, Fig. 1) is based on dual-log transformed linear regression analysis. The minimum temporal difference between their correlation and perfect correlation is 8.9 h (at 2 months), which is problematic given that they used time intervals of 2, 3, and 13 months (month being 30.44 ± 0.84 days). It is not possible to assess probability with such accuracy without knowing precise times (within hours) of treatment and sera withdrawal. Moreover, linear regression with three data points produces one residual degree of freedom ($df_{residual}$) for variance statistic (F) determination. F-probability distributions based on such low $df_{residual}$ are unreliable. Also, if antibody titers are proposed to peak and decay, then dual-axis data transformation is not preferred. A daily decay constant k (0.58%/day) based on antibody half-life of 120 days produces an optimal fit (decay-start = 15.4 U/l and base-line = 3 U/l) parameters produce and P value of 0.1 and (heteroscedasticity correction $P = 0.04$, $df_{residual} = 1$).

Since each DR molecule can bind a variety of peptides, it is expected that any DR can bind multiple structurally *similar* sites between any two large proteins. In any individual, twelve or less MHC class II isoforms present the immense variety of proteins and polypeptides required for T-helper cell-mediated immunity and tolerance. Also, DR3[-DQ2.5]/DR7[-DQ2.2] and advanced age are risk factors for other autoimmune diseases. The *in silico* analysis of T-cell sites is not useful. MZA holds the Robert A Welch Chair of Chemistry.

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