Endocr (2011) 39:41–47
DOI 10.1007/s12020-010-9410-9

ORIGINAL ARTICLE

Injections of *Clostridium botulinum* neurotoxin A may cause thyroid complications in predisposed persons based on molecular mimicry with thyroid autoantigens

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Received: 6 July 2010 / Accepted: 4 October 2010 / Published online: 9 November 2010

Abstract A woman with Hashimoto's thyroiditis, under replacement L-T4, repeatedly experienced, over a 10-year period, elevations of serum TSH after eyelid injections of *Clostridium botulinum* neurotoxin A (Btx). We hypothesized a link between Btx injections and TSH elevations via molecular mimicry, and aimed to verify our hypothesis. Using an *in silico* approach, we searched first for amino acid sequence homology between Btx and thyroid autoantigens, and next for HLA binding motifs within homologous segments. We found that (i) Btx and thyroid autoantigens share amino acid sequence homology; (ii) some homologous regions contain epitopes of both Btx and thyroid autoantigens; (iii) some of such regions contain HLA-DR3 and/or HLA-DR7 binding motifs, which predominate over other HLA-DRs. This is relevant because the patient's HLA-DR haplotype was DR3/DR7. In conclusion, clinical and bioinformatics data suggest a possible pathogenetic link between Btx and autoimmune thyroid diseases. Considering the wide and increasing medical and dermocosmetic use of Btx, and the frequently subclinical course of autoimmune thyroid diseases, we think that thyroid "complications" may pass frequently undetected in Btx-treated persons.

Keywords *Clostridium botulinum* neurotoxin A · Autoimmune thyroid diseases · Thyrotropin · Molecular mimicry · Amino acid sequence homology

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Introduction

Clostridium botulinum neurotoxin A (Btx) is an efficacious therapy for several neurologic conditions (muscular disorders, hypersecretory and certain pain syndromes) [1]. More recently, it has been widely used in dermatology, mainly for the treatment of wrinkles, with valuable results. For instance, in the United States over 2.5 million non-surgical cosmetic procedures with Btx have been performed in 2009 [2]. Used appropriately, it is generally well tolerated, with few side effects [1]. Here we report a case of a thyroid complication that was clinically asymptomatic because the patient was already under replacement therapy.

Case report

A minute 70-year old Caucasian woman came to our observation (time 113 months in Fig. 1) reporting intermittent

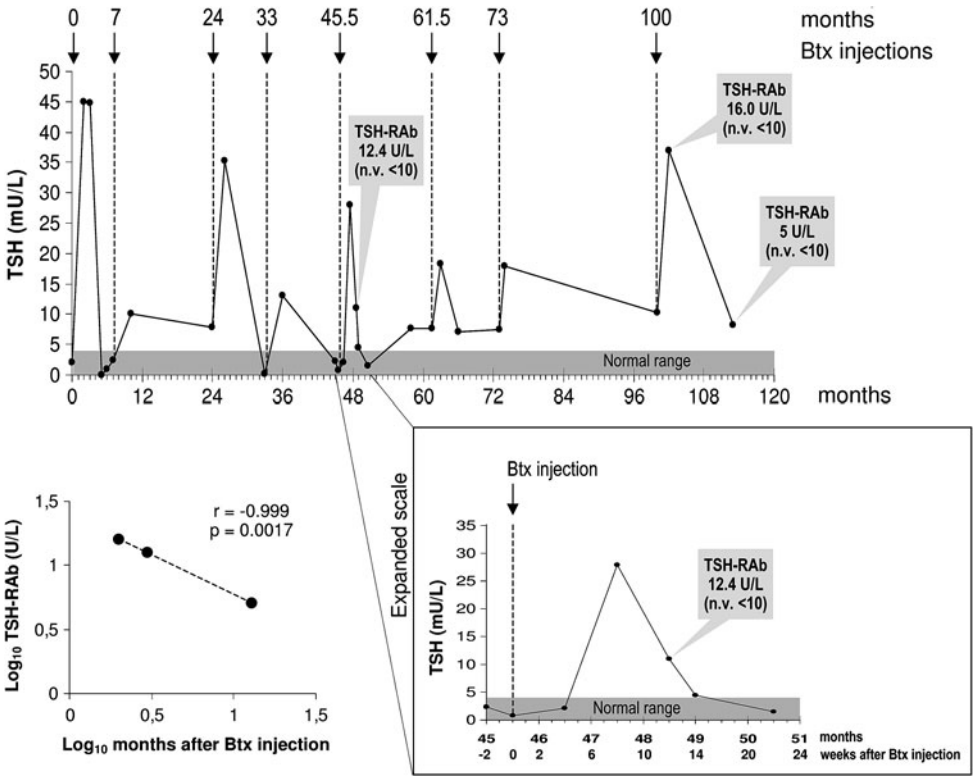


Fig. 1 Serum TSH levels of the patient from the time of first eyelid injection of Btx (month 0) through our observation (month 113). On one occasion, TSH was measured repeatedly after one such injection (*right inset*). Levels of anti-TSH-R antibodies (TSH-RAb) at specific time points are shown in gray callouts. Note the very high correlations between available TSH-RAb levels and months elapsed after the injection preceding the corresponding time point when TSH-RAb was assayed (*left inset*). Not shown is the equivalent correlation concerning serum TSH ($r = -0.611$, $P = 0.007$, see text). *Btx Clostridium botulinum* neurotoxin A, *n.v.* normal values

elevations in serum TSH over the last 10 years in spite of uninterrupted substitutive therapy with L-T4 (50 μg/day). She had been diagnosed primary hypothyroidism due to Hashimoto's thyroiditis (HT) many years prior to our observation and put on L-T4 therapy. A few days prior to observation (month 113), she had measured serum TSH (8.2 mU/l, normal values 0.3–4.0), FT3 (4.7 pmol/l, nv 3.0–6.7), FT4 (16.4 pmol/l, normal values 12–22), thyroglobulin antibodies (TgAb, 717 U/ml, normal values <100), thyroperoxidase antibodies (TPOAb, 1,790 U/ml, normal values <100), and serum TSH receptor antibodies (TSH-RAb) (5 U/l, normal values <10). These data confirmed HT. The patient did not return for further evaluation.

In view of the TSH pattern over time and in silico findings that are described below, and because the patient was lost to follow-up, it was important to obtain as many data as possible on TSH-RAb. We were able to find two small frozen aliquots of sera corresponding to months 48.5 and 102 (Fig. 1). TSH-RAb in these two sera resulted abnormally elevated (12.4 and 16.0 U/l, respectively; normal values <10).

Careful history disclosed that she never took drugs interfering with L-T4 gastrointestinal absorption [3]. The only treatment was cosmetic, namely, eyelid injection of 200 U Btx (Dysport, Porton Products, UK) for essential bilateral blepharospasm. The first injection had occurred 10 years prior to our observation (time 0 in Fig. 1). From the charts she displayed, we were able to reconstruct the consistent pattern shown in Fig. 1. In one occasion, the patient had undergone frequent TSH assays just prior to a Btx eyelid injection and within the subsequent 6 months. To better appreciate TSH changes, data are plotted also on an expanded scale (Fig. 1, right inset). The pattern consists of a transient elevation of serum TSH following each Btx injection, with a peak between the second and the third month post-injection. TSH elevation was variable (lowest peak = 10 mU/l; highest peak = 45 mU/l). Both log 10-transformed serum TSH levels and log 10-transformed TSH-RAb correlated negatively at highly significant levels with the time (in log 10-transformed months) elapsed after each preceding Btx injection (TSH, $r = -0.611$, $P = 0.007$, data not shown; TSH-RAb, $r = -0.999$, $P = 0.0017$, left inset of Fig. 1). This pattern suggests that changes of TSH and TSH-RAb over the 10-years follow-up were not random fluctuations.

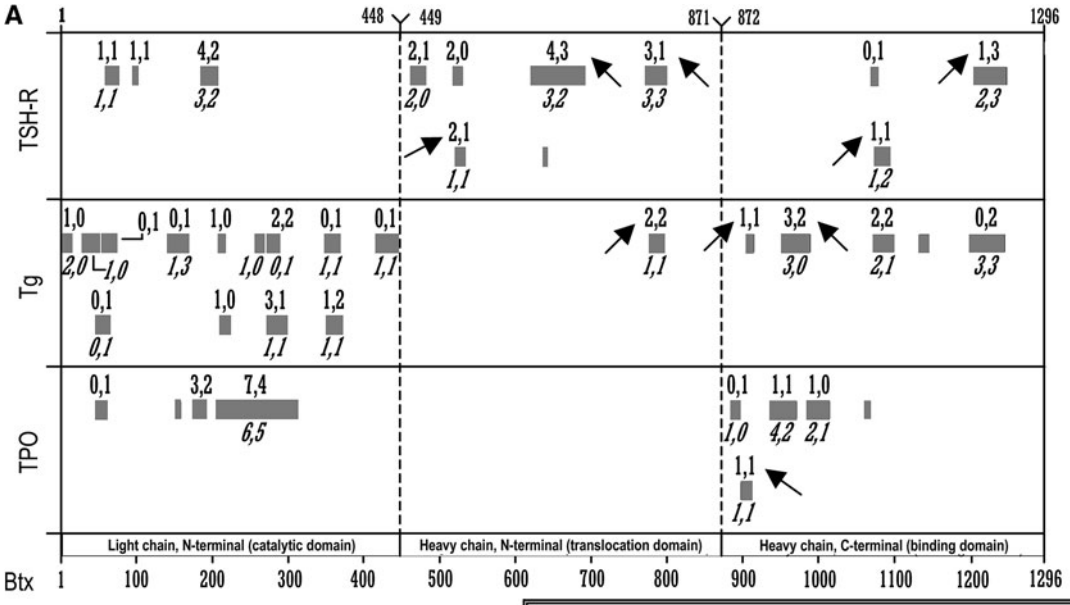
In addition, TSH-RAb were measurable in 3/3 occasions they were assayed (Fig. 1). Particularly, TSH-RAb were elevated when assayed shortly (2 months) after BTx injections and within normal limits when assayed longer (5 months) after Btx injection. Evident is the positive correlation with serum TSH: the highest TSH-Ab value coincided with a TSH peak, while the lowest with a low TSH level, and the intermediate with an intermediate TSH level.

Results

As we are interested in the pathogenesis of autoimmune thyroid diseases (AITD) as the result of interactions between genes and environment (particularly, class II HLA-DR genes and microbic proteins with local amino acid sequence resemblance to thyroid autoantigens) [4, 5], we wished to know the HLA-DR haplotype of this patient and look for amino acid sequence homologies between Btx and thyroid autoantigens (thyrotropin receptor [TSH-R], thyroperoxidase [TPO], and thyroglobulin [Tg]). Subsequently, we searched the retrieved microbial and homologous human sequences for peptide binding motifs of T cell receptor/HLA molecules. HLA phenotyping by the standard method of microlymphotoxicity [6] on blood lymphocytes revealed an HLA-DR3/DR7 haplotype in our patient. By the said computer-assisted search, we found 12 segments of Btx-homologous to TSH-R, 19 homologous to Tg, and nine homologous to TPO (Fig. 2a). Of these segments, 11/12, 7/19, and 2/9, respectively, included en bloc or most part of known epitopic regions of the respective thyroid autoantigen [7]. Amino acid identity ranged 18–66% (TSH-R), 25–60% (Tg), and 20–100% (TPO), while overall homology (that is, identity + chemical similarity) ranged 41–100% (TSH-R), 35–77% (Tg), and 36–100% (TPO).

For TSH-R, homologous segments were located mainly in the heavy chain of Btx, particularly in the translocation domain. Homologous segments of Tg lied mainly within the catalytic domain of Btx, while homologous parts of TPO were almost equally distributed between the catalytic and the binding domain. Except for a single segment of Tg, only TSH-R had regions matching the translocation domain of Btx.

DR3, DR4, and DR7 binding motifs were those most represented in the segments shared between Btx and thyroid autoantigens; DR5 and DR6 motifs were the least represented (Fig. 2b). At least one copy of HLA-DR3 binding motifs shared by the Btx segment and its homologous thyroid autoantigen segment was present in 9/12



B

	DR1		DR2 (15)		DR3		DR4		DR5 (11,12)		DR6 (13,14)		DR7		DR8		DR9	
	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T	B	T
TSH-R	3	4	4	11	21	15	11	20	0	1	0	2	16	14	3	4	7	3
Tg	4	3	9	4	17	19	20	18	0	0	6	0	18	14	16	7	4	14
TPO	5	7	3	7	13	10	14	7	1	2	1	0	14	9	9	8	10	4
Total	12	14	16	22	51	44	45	45	1	3	7	2	48	37	28	19	21	21

Fig. 2 **a** Schematic representation of the protein segments of Btx that are homologous to human TSH-R, Tg, or TPO. *Arrows* indicate segments that overlap with known epitopes of Btx and that contain at least one HLA-DR3 binding motif in the Btx sequence overlapping with Btx epitope(s) and in the homologous sequence of the thyroid autoantigen. The *arrow-labeled* segments are: Btx 521–533, 621–692, 773–799, 1073–1090, 1205–1244 (homologous to TSH-R sequences 149–161, 586–656, 645–671, 170–187, and 152–188, respectively); Btx 777–796, 906–914, 952–988 (homologous to Tg segments 98–117, 1856–1864, and Btx 315–351, respectively); 897–910 (homologous to the TPO segment 104–117). The position of the domains is noted in the 1296-residue long Btx. Further explanations are given on the *left* of (**b**). **b** Number of HLA-DR binding motifs in the homologous segments of Btx (columns labeled B) and thyroid autoantigens (columns labeled T). Columns for HLA-DR3 and HLA-DR7 are highlighted in *gray* because they were the HLA-DR loci possessed by the patient

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Btx      621 VSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGAL 656
             V T + +A + +   ++   + + N  Y
TSH-R    586 VLTLNIVAFVIVCCCHVKIYITVRNPQYNPGDKDTK 621

Btx      657 IFSGAVILLEFIPEIAIPVLGTFALVSYIANKVLTV 692
             I     +L+ F   I +  +  +AL + +   ++TV
TSH-R    622 IAKRMAVLI-FTDFICMAPISFYALSAILNKPLITV 656
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Btx      621 VSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGAL 656
             V T + +A + +   ++   + + N  Y
TSH-R    586 VLTLNIVAFVIVCCCHVKIYITVRNPQYNPGDKDTK 621

Btx      657 IFSGAVILLEFIPEIAIPVLGTFALVSYIANKVLTV 692
             I     +L+ F   I +  +  +AL + +   ++TV
TSH-R    622 IAKRMAVLI-FTDFICMAPISFYALSAILNKPLITV 656
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Fig. 3 Amino acid sequence alignment of the homologous segments 621–692 of Btx and 586–656 of TSH-R. The *symbol plus* (+) indicates chemical similarity between two aligned amino acids. HLA-DR3 (*top panel*) or HLA-DR7 (*bottom panel*) binding motifs in both sequences are typed in *bold-face*. HLA-DR3 motifs are L/I/F/M/V, X, X, D, X, K/R/E/Q/N, X, L, Y/L/F or L/I/F/M/V, X, X, D, X, K/R/E/Q/N or F/I/L/V/Y, X, X, D/N/Q/T; HLA-DR7 motif is F/I/L/V/Y, X, X, X, X, N/S/T (see [6])

(75%) matches concerning TSH-R, 7/19 (37%) matches concerning Tg, and 4/9 (44%) matches concerning TPO; the corresponding numbers for HLA-DR7 motifs were 7/12 (58%), 8/19 (42%), and 4/9 (44%). One example homology, which includes both HLA-DR3 and HLA-DR7 motifs, is presented in Fig. 3.

By searching the IEDB (Immune Epitope Database and Analysis Resource) [7], we identified 51 linear and three discontinuous epitopes of Btx (as of December 30, 2009). With a single exception, all belong to the heavy chain. Some linear epitopes, but no discontinuous epitope, overlapped totally or largely with the thyroid autoantigen-like sequences of Btx. These segments, which are indicated by arrows in Figs. 2a and 4, show the Btx-like homologous segments of TSH-R ($n = 9$), Tg ($n = 11$), and TPO ($n = 5$) that share at least one HLA-DR3 or DR7 motif, and their position relative to known epitopes of each thyroid autoantigen. Noteworthy, for TSH-R all nine segments lie within epitope-containing parts of the autoantigen, or overlap largely with them; for Tg and TPO these segments are 4/11 and 2/5, respectively.

Discussion

Taken together, the patient's history, her HLA-DR haplotype, and our *in silico* data support the hypothesis of a causal link between Btx injections and transient elevations of serum TSH via molecular mimicry between Btx and TSH-R. As shown in Table 1, with a score of nine, our patient matched the cut-off score of nine required in the Naranjo scale [8] to attribute definitely adverse events (transient TSH increase, in our case) to a pharmacologic intervention (intramuscular Btx injection).

Table 1 Naranjo scale of causal correlation between drug intake and adverse reactions

1. *Q. Are there previous conclusive reports on this reaction ?*
   A. Yes (+1), **No (0)**, Do not know or not done (0)
2. *Q. Did the adverse event appear after the suspected drug was given ?*
   A. **Yes (+2)**, No (−1), Do not know or not done (0)
3. *Q. Did the adverse reaction improve when the drug was discontinued or a specific antagonist was given ?*
   A. **Yes (+1)**, No (0), Do not know or not done (0)
4. *Q. Did the adverse reaction appear when the drug was readministered ?*
   A. **Yes (+2)**, No (−2), Do not know or not done (0)
5. *Q. Are there alternative causes that could have caused the reaction ?*
   A. Yes (−1), **No (+2)**, Do not know or not done (0)
6. *Q. Did the reaction reappear when a placebo was given ?*
   A. Yes (−1), No (+1), **Do not know or not done (0)**
7. *Q. Was the drug detected in any body fluid in toxic concentrations ?*
   A. Yes (+1), No (0), **Do not know or not done (0)**
8. *Q. Was the reaction more severe when the dose was increased, or less severe when the dose was decreased ?*
   A. Yes (+1), No (0), **Do not know or not done (0)**
9. *Q. Did the patient have a similar reaction to the same or similar drugs in any previous exposure ?*
   A. **Yes (+1)**, No (0), Do not know or not done (0)
10. *Q. Was the adverse event confirmed by any objective evidence ?*
    A. **Yes (+1)**, No (0), Do not know or not done (0)

The score for each answer is shown in parentheses, and it has been typed boldface in our case

After local injection, Btx undergoes systemic diffusion [9], with subsequent detection of anti-Btx serum antibodies, which may be neutralizing in nature, in approximately 50% of treated persons [10, 11]. Owing to molecular mimicry between Btx and TSH-R, some anti-Btx antibodies can recognize and bind to TSH-R. Thus, transient elevations of TSH in blood that follow each Btx injection might be due to elicitation of anti-TSH-R Ab that "neutralize" to some degree the TSH-R signaling (TSH-R blocking antibodies, TSHR-BAb). Not only, as noticed at the end of the "Case Report" section, linear regression analysis suggested that serum levels of both TSH and TSH-RAb did not change randomly over time, but each transient elevation of TSH lasted approximately 1–3 months (Fig. 1). This duration of 1–3 months coincides with the half-life of circulating antibodies of the IgG class, such as TSHR-BAb [12, 13]. In sum, there would be an acquired, transient TSH resistance that overlaps the biochemical picture of increased serum TSH coexisting with normal serum FT4 observed in the heterozygotes with genetic TSH resistance, viz impaired TSH-R signaling due to a monoallelic inactivating mutation of TSH-R [14–16].

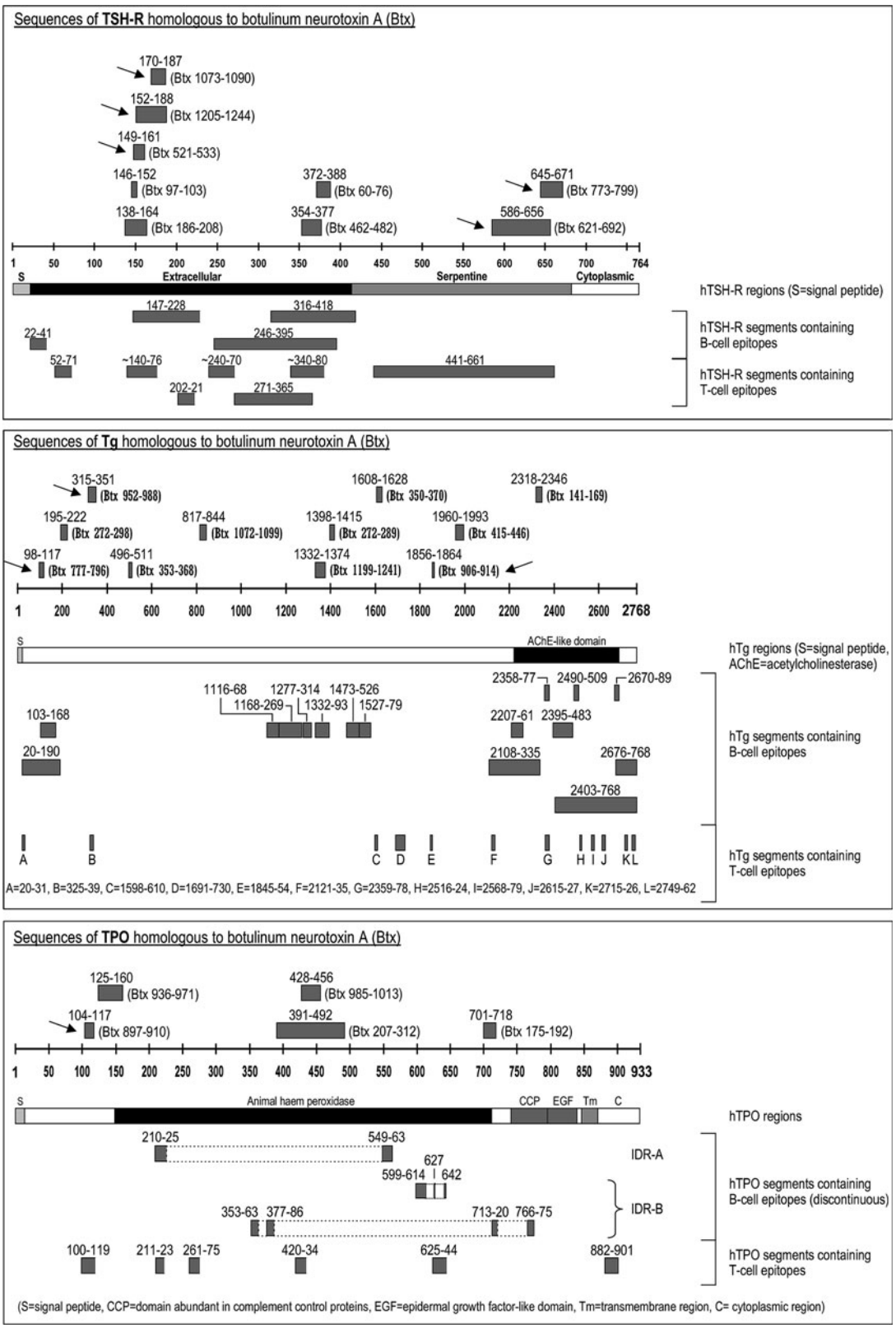

Fig. 4 Segments of the three human thyroid autoantigens (TSH-R, Tg, and TPO) containing HLA-DR3 and/or HLA-DR7 binding motifs that are homologous to Btx segments also containing HLA-DR3 and/or HLA-DR7 binding motifs. The *bottom part* of each panel shows the position of epitopes in each thyroid autoantigen. *Arrows* indicate Btx-homologous segments that, in addition to the HLA-DR3 and/or DR7 binding motifs, also contain the epitopes of Btx specified in the legend for Fig. 2

This situation in which hyperthyrotropinemia is associated with normothyroxinemia ("compensated hypothyroidism"), as opposed to the expected hypothyroxinemia, has still an "unknown underlying mechanism" [15]. However, we do offer a plausible explanation that applies both to the acquired form of TSH resistance due to TSHR-BAb (as in our case) and the genetic form. As well known, TSH production and release in circulation is regulated through a negative feedback exerted by T4 and T3 on the pituitary (long feedback) and the hypothalamus (ultra-long feedback). In addition, but less well appreciated, TSH is regulated by TSH itself at the level of both the hypothalamus (short feedback) and the pituitary (ultra-short feedback) [17]. Indeed, TSH-R is expressed both in the anterior pituitary [17] and in the hypothalamus [18, 19]. As pituitary is outside the blood–brain barrier, circulating IgG have direct access to the pituitary TSH-R. In addition, thyroid autoantibodies of patients with AITD have also access to the hypothalamic TSH-R, because thyroid autoantibodies are long known to be detectable in the cerebrospinal fluid [20].

In our patient, the T4 decrease caused by the peripheral action of TSHR-BAb on the thyroid-expressed TSH-R is normalized by the T4 supplementation. The normal TSH levels associated with the T4 therapy-normalized FT4 concentration are perturbed by the lack of TSH inhibition at the pituitary and hypothalamus level because of the TSH-R hypofunction caused by the TSHR-BAb. The final result is, therefore, increased serum TSH. The decrease of TSHR-BAb some time after Btx administration would then allow the progressive restoration of the equilibrium among the various feedback loops and, consequently, the return to normal TSH levels. The approximate 2-month lag-time between Btx injection and TSH peak, the approximate 1–3 months required for the increased TSH to return at pre-injection levels, the increase in serum TSH-RAb after Btx injections, and the known approximately 4-week long half-life of circulating IgG, are consistent with our explanation.

Autoimmunity results from a complex interplay between genes and environment. Among genetic factors, HLA plays a major role. In the case of AITD in Caucasians, HLA-DR3, HLA-DR4, and HLA-DR5 are known risk factors for HT, while HLA-DR3 is a risk factor for Graves' disease [21–25]. This agrees with the autoimmune disease of our patient (HT), and with her HLA-DR haplotype (DR3/DR7), because HLA-DR3, HLA-DR4, and HLA-DR7 binding motifs were the most frequent in the segments shared by Btx and thyroid autoantigens.

HLA class II molecules are critical in the initiation of the adaptive immune response, as they present peptide antigens to T cells [4, 5]. $CD4^+$ T cells, through their T cell antigen receptor, recognize small linear peptides only when those peptides are attached to the binding groove of an appropriate HLA class II molecule. In general, the antigens presented by class II molecules are derived from the degradation of pathogens.

Unfortunately, we lost the patient to follow-up and could not measure, with in vitro bioassay, TSHR-BAb prospectively with respect to Btx injections. TSH-RAb had been assayed, as routinely is done, with the classic radioreceptor method, which is a method unable to distinguish between TSH-R-stimulating and TSH-R-blocking antibodies. Circulating TSHR-BAb (also known as TBII [TSH binding inhibitory immunoglobulins]) occur naturally in a number of hypothyroid patients with HT, and are distinct from the TSHR-SAb which occur naturally in most Graves' disease patients [26]. Importantly, TSH epitopes recognized by the two distinct types of TSH-RAb do not coincide [26]. Epitopes for TSHR-BAb are contained in the region 270–395 of TSH-R, which we found to contain two segments (aa. 354–377 and 372–388) similar to two segments of Btx (Fig. 4, top panel). Noteworthy, it has been experimentally shown that antibodies binding to the segment 381–385 of TSH-R are "quite effective in blocking TSH-R stimulation by TSH" [27]. The total number of DR3 binding motifs were 2 in these two regions of TSH-R, and 3 in the matched regions of Btx; the corresponding total number for DR7 motifs were 1 and 3. Positiveness for TPOAb and TgAb does not contradict our hypothesis, because these are fairly persistent Ab that, especially in HT patients, can be detected at extremely high levels. Accordingly, the 1,790 and 717 U/ml measured in our patient 13 months after the Btx injection could represent a fall from much greater levels.

As Btx injections are also used for treatment of the upper lid retraction of thyroid eye disease (TED) and because TSH-R is also expressed in the orbital tissues [28], our findings could explain the reported aggravation of TED following such treatment [29].

As an implication of our observation, we suggest that thyroid function and autoantibodies should be investigated before and after treatment with Btx. Indeed, considering the frequently subclinical nature of AITD, triggering of HT (or aggravation from euthyroidism to subclinical hypothyroidism) in a genetically predisposed individual can remain clinically silent. In addition, prospective studies are warranted to test whether carriers of certain HLA haplotypes are at high risk of Btx-elicited thyroid complications.

Materials and methods

Amino acid sequence homologies between Btx and thyroid autoantigens (TSH-R, TPO, and Tg) were searched using the NCBI (National Center for Biotechnology Information) software BLAST (Basic Local Alignment Search Tool)

[30]. The search for peptide binding motifs of T cell receptor/HLA-DR molecules [4] in the microbial and homologous human sequences were performed using the HLA ligand/motif database [31].

References

1. R. Hackett, P.C. Kam, Med. Chem. **3**, 333–345 (2007)
2. American Society for Aesthetic Plastic Surgery, News release on Statistics, Surveys and Trends in Aesthetic Medicine in 2009. http://www.surgery.org/media/news-releases/despite-recession-overall-plastic-surgery-demand-drops-only-2-percent-from-last-year (checked September 17, 2010) (2010)
3. S. Benvenga, L. Bartolone, M.A. Pappalardo, A. Russo, D. Lapa, G. Giorgianni, G. Saraceno, F. Trimarchi, Thyroid **18**, 293–301 (2008)
4. S. Benvenga, L. Santarpia, F. Trimarchi, F. Guarneri, Thyroid **16**, 225–236 (2006)
5. F. Guarneri, S. Benvenga, Curr. Opin. Endocrinol. Diabetes Obes. **14**, 398–409 (2007)
6. National Institute of Health Transplantation and Immunology Branch Staff, in *Manual of tissue typing techniques, DHEW publication no. 80-545*, ed. by T. Ray (National Institute of Health, Bethesda, 1979)
7. B.Peters, J.Sidney, P.Bourne, H.H.Bui, S.Buus, G.Doh, W.Fleri, M.Kronenberg, R.Kubo, O.Lund, D.Nemazee, J.V.Ponomarenko, M.Sathiamurthy, S.Schoenberger, S.Stewart, P.Surko, S.Way, S.Wilson, A.Sette, *PLoS Biol*. **3,** e91 (2005) Database freely available at http://www.immuneepitope.org, accessed on 28 October 2009
8. C.A. Naranjo, U. Busto, E.M. Sellers, P. Sandor, I. Ruiz, E.A. Roberts, E. Janecek, C. Domecq, D.J. Greenblatt, Clin. Pharmacol. Ther. **30**, 239–245 (1981)
9. European Medicines Agency 2003 Referral on Botox – Annex III. Available at http://www.ema.europa.eu/pdfs/human/referral/botox/426003en.pdf, accessed on May 1, 2010
10. D. Dressler, Nervenarzt **79**(Suppl 1), 36–40 (2008)
11. H. Göschel, K. Wohlfarth, J. Frevert, R. Dengler, H. Bigalke, Exp. Neurol. **147**, 96–102 (1997)
12. Z. Kraiem, B.Y. Cho, O. Sadeh, M.H. Shong, P. Pickerill, A.P. Weetman, Clin. Endocrinol. (Oxf) **37**, 135–140 (1992)
13. S.B. Feingold, J. Smith, J. Houtz, E. Popovsky, R.S. Brown, J. Clin. Endocrinol. Metab. **94**, 4742–4748 (2009)
14. S. Refetoff, J. Endocrinol. Invest. **26**, 770–779 (2003)
15. S. Refetoff, J. Chin. Med. Assoc. **66**, 441–452 (2003)
16. A. Nicoletti, M. Bal, G. De Marco, L. Baldazzi, P. Agretti, S. Menabò, E. Ballarini, A. Cicognani, M. Tonacchera, A. Cassio, J. Clin. Endocrinol. Metab. **94**, 4187–4194 (2009)
17. M.F. Prummel, L.J. Brokken, W.M. Wiersinga, Thyroid **14**, 825–829 (2004)
18. P. Crisanti, B. Omri, E. Hughes, G. Meduri, C. Hery, E. Clauser, C. Jacquemin, B. Saunier, Endocrinology **142**, 812–822 (2001)
19. J. Bockmann, C. Winter, W. Wittkowski, M.R. Kreutz, T.M. Bockers, Biochem. Biophys. Res. Commun. **238**, 173–178 (1997)
20. R. Hall, S.G. Owen, Br. Med. J. **2**, 710–711 (1960)
21. S.M. McLachlan, B. Rapoport, in *Werner & Ingbar's the thyroid: a fundamental and clinical text*, 8th edn., ed. by L.E. Braverman, R.D. Utiger (Lippincott Williams & Wilkins, Philadelphia, 2000)
22. Y. Ban, Y. Tomer, Clin. Dev. Immunol. **12**, 47–58 (2005)
23. Y. Tomer, A. Huber, J. Autoimmun. **32**, 231–239 (2009)
24. E.M. Jacobson, A. Huber, Y. Tomer, J. Autoimmun. **30**, 58–62 (2008)
25. M. Dittmar, G.J. Kahaly, Thyroid **15**, 239–250 (2005)
26. L.D. Kohn, N. Harii, Autoimmunity **36**, 331–337 (2003)
27. B.R. Smith, J. Sanders, J. Furmaniak, Thyroid **17**, 923–938 (2007)
28. P. Perros, A.J. Dickinson, in *Werner & Ingbar's the thyroid: a fundamental and clinical text*, 8th edn., ed. by L.E. Braverman, R.D. Utiger (Lippincott Williams & Wilkins, Philadelphia, 2005)
29. A.R. Harrison, J.P. Erickson, Ophthal. Plast. Reconstr. Surg. **22**, 397–398 (2006)
30. S.F.Altschul, T.L.Madden, A.A.Schäffer, J.Zhang, Z.Zhang, W.Miller, D.J.Lipman, *Nucleic Acids Res*. **25,** 3389–3402 (1997) (software freely available at http://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 28 February 2009)
31. M. Sathiamurthy, H.D. Hickman, J.W. Cavett, A. Zahoor, K. Prilliman, S. Metcalf, M. Fernandez Vina, W.H. Hildebrand, Tissue Antigens **61**, 12–19 (2003)