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LETTER TO THE EDITOR

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

Edvina Gregoric · Jurji Avramovic Gregoric · Fabrizio Guarneri · Salvatore Benvenga

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We thank Atassi and Deitiker [1] for the opportunity to give some clarifications.

Their references 2 and 3 (the latter published after our paper) report epitopes targeted by protective antibodies against *Clostridium botulinum* neurotoxin A (Btx), different from our epitopes. However, because our patient was not immunoresistant to 10-year duration of Btx injections, chances are she had non-neutralizing Btx-antibodies.

Contiguity of four or more identical aminoacids is not necessary for cross-reaction, and the molecular mimicry hypothesis cannot be rejected on this basis. For example, the peptide YVLEGTLTA (aa 165–173 of *Borrelia burgdorferi* OspA) experimentally cross-reacts with YVIEGTSKQ (aa 332–340 of human LFA-1) [2]. We agree that only experimental evidence is definitive, but our patient did not return subsequently making impossible further tests with synthetic peptides, other Btx formulations or placebo.

We could not find data on the concentration of flagellins in Dysport®, nor about possible contamination of other Btx products by flagellins. It is therefore impossible to say whether administration of a different Btx product would make a difference. However, it seems unlikely that flagellins in Dysport® can trigger thyroid autoimmunity. Available data [3] suggest that they interact with TLR-5, perhaps acting as adjuvants but not causing anti-flagellin response. Moreover, while many researches detected Btx antibodies, none reported flagellin antibodies following Btx injections.

Statistical significance of homologies concerning short peptides not always correlates to biologic significance. For example, an *E* value of 23 (largely statistically insignificant) is obtained when comparing the aforementioned *Borrelia* epitope YVLEGTLTA against itself. Although our proposed antigen mimicry needs experimental testing, it cannot be ruled out based on statistics. We never mentioned discontinuous epitopes because we believe that, based on our analysis, they are not involved in this case.

Concerning data in our Fig. 1 (left inset), in principle it is correct that the more are the data the more accurate and reliable are correlations. The graphic, based on the only three values available, is meant to show a consistent trend to decrease of TSHR-Ab across months after Btx injection. However, we could not ignore the resulting perfectly linear statistically significant inverse relationship. Finally, the mechanism of antigen presentation, detailed in literature [4], explains HLA-related predisposition to specific autoimmune diseases through molecular mimicry. Peptide binding

E. Gregoric
Department of Nuclear Medicine, Hospital Izola, Izola, Slovenia

E. Gregoric · J. A. Gregoric · S. Benvenga
Master of Childhood, Adolescent and Women's Endocrine Health, Università di Messina, 98125 Messina, Italy

J. A. Gregoric
Department of Internal Medicine, Hospital Izola, Izola, Slovenia

F. Guarneri
Istituto di Dermatologia, Università di Messina, 98125 Messina, Italy

S. Benvenga
Sezione di Endocrinologia, Dipartimento Clinico Sperimentale di Medicina e Farmacologia, Università di Messina, Messina, Italy

S. Benvenga
Programma di Endocrinologia Molecolare Clinica, AOU Policlinico G. Martino, 98125 Messina, Italy

F. Guarneri (✉)
Viale Annunziata, Residence dei Fiori, villa 7, 98168 Messina, Italy
e-mail: f.guarneri@tiscali.it

specificity of an MHC molecule, defined by the corresponding HLA gene, determines which peptide of a protein is presented to T cells to achieve immune response. When the peptide mimics an autoantigenic epitope, specific autoimmunity can be triggered. Search for HLA binding motifs confutes the claim that "any DR can bind multiple structurally similar sites between any two large proteins". Moreover, keeping in mind that our patient had DR3 and DR7 loci, we found DR3/DR7 binding motifs in immunologically relevant sequences, i.e., antigenic segments of Btx and homologous, autoantigenic epitope-containing sequences of TSH-R. Like any risk factor, an HLA genotype can predispose to multiple autoimmune diseases, because the corresponding MHC molecule can present epitopes sharing its binding motif, but belonging to different autoantigens [4].

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