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Influences of HLA DRB1, DQA1 and DQB1 on T-cell recognition of epitopes and of larger regions of the botulinum neurotoxin molecule

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ABSTRACT

Previously, we have examined the proliferative responses of T-cells from 25 patients and 8 controls to 32 light chain (L1-L32) and 60 heavy chain peptides (N1-N29, C1-C31) representing the entire clostridium botulinum neurotoxin type A (BoNT/A)[OM1-OM3]. In the current work, these T-cell responses were analyzed in the context of the patients HLA-DRB1, DQA1 and DQB1 variation. There were strong associations between the DQA1*01:02 and its derived haplotypes and cumulative T-cell proliferative responses. With or without HLA based differentiation the responses showed marked correlation. Inter-epitope correlation of responses demonstrably associated with particular regions (peptides N1-N29) peaking in the region covered by of N18-29. A second region of higher correlation was observed close to the carboxyl terminal of the heavy chain. Region N15 to N29 was found to have a significantly lower Stimulation Indices when DRB1*01:01-DQA1*01:01-DQB1*05:01 was present. This pattern was also evident in the HLA analysis where DQA1*01:02 associations were collectively most significant in the N1-N13 & C16-C31 region. Responses in these regions correlated well with one another. HLA-specific correlation analysis revealed that DQA1*01:01 bearing haplotype had the strongest inter-epitope correlations despite having a generally negative association with simulation indices. Structural and immunogenic implications of these findings are discussed.

1. Introduction

Clostridium botulinum neurotoxins (BoNTs) (MW, 150 kDa) are a group of eight serotypes (A through H) of the most toxic substances known. Consisting of a heavy (H) chain (MW, 100 kDa) and a light (L) chain (MW, 50 kDa), the toxin ultimately blocks acetylcholine release from nerve terminals at the neuromuscular junction [1–3]. BoNT/A (and to a lesser degree BoNT/B) is increasingly being used to treat a variety of neuromuscular disorders [4] The neuro-paralyzing activity of BoNTs is reversible and requires repeated injections at 3–6-month intervals.

Treatment of patients with a BoNT/A can cause them to develop neutralizing (blocking) antibody (Ab) against the toxin [5–9]. The incidence of blocking Abs that interfere with treatment has decreased in relative frequency after the introduction of the current BOTOX® (Allergan) [10,7,9]. Studies on T-cell responses demonstrated that BoNT/A-specific peripheral blood lymphocytes (PBLs) are elevated in about 70% of patients with neuromuscular disorders (e.g. spasmodic torticollis) treated with BoNT/A [11]. This response elevation is accompanied by alteration from naïve T lymphocyte subpopulation to effector/memory subpopulation [11,12]. Immune responses are controlled by genes [13] both of the MHC [14] and by non-MHC genes [15]. In our studies of PBL responses to BoNT/A of treated-patients we also examined the high-resolution HLA DRB1, DQA1 and DQB1 genetics for treated patients. However, based on predicted control frequencies for the regional population no allele or haplotypes demonstrated significant linkage to BoNT/A T-cell responses (Data not published).

Subsequently the submolecular localization of treated-patient T-cell recognition was examined. The entire BoNT/A molecule was represented by 32 peptides (L1-L32) encompassing the light chain and 60 peptides (N1-N29 and C1-C31) encompassing the entire heavy chain

Abbreviations: BoNT/A, *Clostridium botulinum* neurotoxin type A; LC, light chain; H_C, carboxyl terminal domain of the heavy chain; H_N, amino terminal domain of the heavy chain; HLA, Human leukocyte antigen; DR, HLA DR-region (class II MHC) isoform composed of $\alpha\beta_x$; DRB1, the primary DR β-encoding gene of HLA-DR region; DQ, isoform composed of $\alpha_{1\beta1}$ HLA DQ region primary gene products; DQA1, the gene encoding the primary DQα subunit; DQB1, the gene encoding the primary DQβ subunit; TVNB, a comparison of a specific type bearing group versus all other (type non-bearers) patients; SI, simulation index; PBL, peripheral blood lymphocytes

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Table 1
HLA DR-DQ genotype for 25 BoNT/A treated individuals used in this study.

	Deduced	DRB1 alleles	DQA1 alleles	DQB1 alleles
1	DR3-DQ2.5/DR1.3-DQ7.5	*01:03/*03:01	*05:01/*05:05	*02:01/*03:01
2	DR9-DQ7.6/DR11-DQ7.5	*09:01/*11:01	*05:05/*06:01	*03:01 (hz)
3	DR7-DQ2.2/DR8-DQ4.4	*07:01/*08:01	*02:01/*04:01	*02:02/*04:02
4	DR15-DQ6.2/DR8-DQ4.4	*15:01/*08:01	*01:02/*04:01	*06:02/*04:02
5	DR15-DQ6.2/DR4.5-DQ8.3	*15:01/*04:05	*01:02/*03:03	*06:02/*03:02
6	DR1-DQ5/DR15-DQ6.2	*01:01/*15:01	*01:01/*01:02	*05:01/*06:02
7	DR4.7-DQ7.3/DR13-DQ6.3	*04:07/*13:01	*03:03/*01:03	*03:01/*06:03
8	DR1-DQ5/DR13.2-DQ6.4	*01:01/*13:02	*01:01/*01:02	*05:01/*06:04
9	DR3-DQ2.5/DR8-DQ4.4	*08:01/*03:01	*05:01/*04:01	*02:01/*04:02
10	DR15-DQ6.2/DR13-DQ6.3	*15:01/*13:01	*01:02/*01:03	*06:02/*06:03
11	DR13.2-DQ6.4	*13:02 hz	*01:02 hz	*06:04 hz
12	DR1-DQ5/DR8-DQ4.4	*01:01/*08:01	*01:01/*04:01	*05:01/*04:02
13	DR1-DQ5/DR11-DQ7.5	*01:01/*11:01	*01:01/*05:05	*05:01/*03:01
14	DR1-DQ5/DR3-DQ2.5	*01:01/*03:01	*01:01/*05:01	*05:01/*02:01
15	DR1-DQ5/DR7-DQ2.2	*01:01/*07:01	*01:01/*02:01	*05:01/*02:02
16	DR7-DQ2.2/DR11-DQ7.5	*07:01/*11:01	*02:01/*05:05	*02:02/*03:01
17	DR15-DQ6.2/DR4.5-DQ8.3	*15:01/*04:05	*01:02/*03:03	*06:02/*03:02
18	DR1-DQ5/DR4-DQ7.3	*01:01/*4:01	*01:01/*03:03	*05:01/*03:01
19	DR15.3-DQ6.2/DR13.2-DQ6.4	*15:03/*13:02	*01:02 hz	*06:02/*06:04
20	DR15-DQ6.2/DR4-DQ8	*15:01/*04:01	*01:02/*03:01	*06:02/*03:02
21	DR15-DQ6.2/DR13-DQ6.3	*15:01/*13:01	*01:02/*01:03	*06:02/*06:03
22	DR13.2-DQ6.4	*13:02 hz	*01:02 hz	*06:04 hz
23	DR15-DQ6.2/DR13-DQ6.3	*15:01/*13:01	*01:02/*01:03	*06:02/*06:03
24	DR15-DQ6.2/DR7-DQ2.2	*15:01/*07:01	*01:02/*02:01	*06:02/*02:02
25	DR15-DQ6.2/DR13-DQ6.3	*15:01/*13:01	*01:02/*01:03	*06:02/*06:03

Hz denotes allelic homozygotes.

[16–18]. In this paper, we examine the association of responses with HLA alleles and haplotypes.

2. Materials and methods

2.1. T-cell analysis

Proliferation analysis of peripheral blood lymphocytes to the 92 BoNT/A peptides (Supplementary material Table 1A and 1B) has been previously described [16–18]. Briefly, proliferation of peripheral blood lymphocytes (PBL) to peptide stimulation is measured by [^{3}H]-thymidine incorporation.

2.1.1. DNA preparation, HLA class II genotyping and haplotype determination

Out of 25 patients enrolled in this study, HLA haplotypes of 23 patients were already determined in previous studies from this laboratory [19]. For the remaining 2 patients, DNA preparation from blood samples, DNA typing and HLA haplotype determination based on published HLA frequency data of US Caucasian population [20,21] were carried out as described previously [19]. Because of the lack of cells from one patient characterization of responses to 46 of 92 peptides were examined.

2.1.2. Analysis of associations of T-cell responses to HLA class-II alleles

Patients were regrouped according to phenotypes and/or genotypes. Groups with more than 5 identical types are analyzed for correlation with the recognition of particular peptides or peptide regions.

Of the 26 patients, 11 patients bore HLA DRB1*15:01-DQA1*01:02-DQB1*06:02 (DR15-DQ6.2), 8 patients bore DRB1*01:01-DQA1*01:01-DQB1*05:01 (DR1-DQ5), and 4 patients each bore the following 5 types: DRB1*13:01-DQA1*01:03-DQB1*06:03 (DR13.1-DQ6.3), DRB1*13:02-DQA1*01:02-DQB1*06:04/09 (DR13.2-DQ6.x), DRB1*11:01-DQA1*05:05-DQB1*03:01 (DR11-DQ7.5), DRB1*08:01-DQA1*04:01-DQB1*04:02 (DR8-DQ4.4), and DRB1*07:01-DQA1*02:01-DQB1*02:01 (DR7-DQ2.2). There were 15 individuals who bore DQA1*0102. They were sorted semi-independently of the DR15-DQ6.2 haplotype and this type was separately examined.

2.2. Analysis of associations of T-cell responses to HLA class-II alleles

All SI data were first normalized to $\mathrm{Log_e}$ SI (LnSI) to compensate for the fact that normal controls frequently had SI distributions that exhibited non-normality with regard to skewing and kurtosis, and normalization reduced the deviancy by 70% and 30%, respectively. Ninety-two peptides could be tested in the patient set subdivided 8 ways for a total of 690 tests performed (46 tests were omitted for HLA DRB1*15:01 because these were redundant with HLA DQB1*06:02 due to the fact a single patient that bore both could not be fully characterized, see above).

Due to asymmetry in data (see above), patient SI values were normalized using the MSEXCEL LN() function. Natural logs of SI values were used for averaging and for Student's *T* test (STT). Mean SI values reported as the mean are converted back (SI^{group} mean = $e^{(groupaverageLog e^{SI})}$) using the MSEXCEL EXP() function. The quantitative test evaluated random probability (P_{STT}) of subdivided means using two-tailed STT that assumed equal variation using the MSEXCEL function TTEST([*type-data*],[*non-bearer data*],2,2). *Type-data* is the collection of SI from patients who bear a given HLA allele, allele group or haplotype; *non-bearer data* is the patient SI of all non-bearers of that same type. Haplotypes are inferred from linkage disequilibrium (LD) between alleles in published databases and within our study. Bonferroni's correction is restricted for parallel tests (in this case 690) and is taken to be standard (0.05)/690. Unless stated otherwise SI difference (ΔSI) is the signed mean difference equals to the $SI^{allelotype}$ minus $SI^{allelotypenon-bearer}$ means. For HLA allelotype (or allele group) comparisons were made to non-bearers of the type (TVNB).

Qualitative statistics [22,p. 483–511] evaluated the chi-squared (χ^2) random probability that two sets of distributions were likely to be significant and used the MSEXCEL CHIDIST function after derivation of χ^2.

The cumulative totals for peptide groupings were obtained by multiplying the average by the number of peptides averages. This was done to avoid biasing the cumulative totals as a result of a single patient (Table 1, #19) for which SI data from L1-L32 and N16-N29 could not be obtained.

Monte Carlo Analyses were performed periodically to look for