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BOTULINUM NEUROTOXIN (BONT/A) AS AN INDUCER OF ACUTE ANTERIOR UVEITIS IN HLA B27 PATIENTS: A LITERATURE REVIEW

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Abstract

Background : *HLA B27 related acute anterior uveitis is a chronic recurrent inflammation of uvea mainly in anterior chamber. The underlying mechanism seems to involves autoimmunity and autoinflammatory. Antigen has been proposed as a trigger, but its precise role is unclear.*
Aim : *We systematically searched evidence for the involvement of antigen, including BoNT/A in triggering HLA-B27 associated disease.*
Methods : *We searched four data base including Pubmed, Ebscohost, Sciencedirect, and Google Scholar. We also review the immunologic response to BoNT/A, its amino acid sequence, and homology with human protein*
Results: *Eight studies reviewed which include the role of antigen in triggering uveitis. Four studies favour autoinflammatory pathway which involves B27 homodimer formation and its interaction with TH-17. Four studies favour autoimmunity pathway which involves molecular mimicry and activation of self reactive CD8+ T-Cells. All of which centralize the role of dendritic cells as antigen presenting cells. Five studies describe immune response to BoNT/A. BoNT/A capable of activating innate and immune system. Using BLAST program from NCBI, we found BoNT/A homology with cytokeratine 8.*
Conclusion : *Two possible pathway on how antigen might trigger HLA B27 AAU. The first is through uveitogenic capacity of the antigen. The second mechanism is through homodimer formation. BoNT/A might induce uveitis in HLA B27 individual through induction of homodimer and activation of self-reactive CD8+ T-Cells due to shared homology with cytokeratine 8 that is extensively expressed in human uvea*

Keyword: *HLA B27, Anterior Uveitis, Botulinum Neurotoxin, Antigen*

INTRODUCTION

Anterior uveitis is the most commonly encountered form of uveitis in daily practice among general ophthalmologist. The most common non-infectious cause of acute anterior uveitis (AAU) is HLA-B27 associated AAU.[1] The prevalence of HLA-B27 in anterior uveitis patients is between 27-50% [2, 3]. The treatment is complex because this disease occur in younger patients, with recurrent nature, and potential ocular as well as systemic complication. The most commonly found ocular complication includes cataract, posterior synechiae, cystoid macular oedema and secondary glaucoma.[4] Around 50-75% patients with HLA B27 uveitis will eventually succumb to spondyloarthropathy.[5] Because of its recurrent nature, understanding what triggers HLA B27 AAU is important.

The role of antigen in the pathogenesis of HLA-B27 associated disease is important, for example an extensive review associates *K. pneumoniae* with this disease[6], and inherent function of HLA class I that is presenting antigen to CD8+ T cells. However their precise role is still unclear. Two mechanism has been proposed; first *autoimmune mechanism,*[7, 8] where HLA class I present homologous antigen to self-protein activating self-reactive CD8+ T-cells. Second mechanism, that is more recently described is *autoinflammatory mechanism.* This is characterised by activations of innate immune cells without specific self-reactive CD8+ T-cells or antibody.[7]

On a case we encountered at our out-patient eye clinic, a 48 years old female presented with severe red eye and diminished visual acuity two weeks prior. One month before initial symptoms, she had underwent botulinum neurotoxin injection in fore-head and periocular area. Further examination revealed that she has history of spondylarthritis and proven HLA-B27 positive through PCR. Prior history of anterior uveitis was denied. She was diagnosed with AAU and treated with topical and oral steroid continued with biologic agents.

Because botulinum neurotoxin (BoNT/A) injection predated uveitis, we suspected that this event triggers the uveitis. BoNT/A is a pharmagologic agent that also embodies antigenic property and capable of eliciting innate immune response through recognition by TLR-2[9], and adaptive immune response by triggering the production of neutralising antibody and proliferation of T-lymphocyte.[10]

To date, there is no single study that associates BoNT/A with HLA-B27 related AAU, making establishment of link between BoNT/A and HLA B27 uveitis difficult. In this review, we aim to describe immunologic response of HLA-B27 individuals stimulated using various antigens and possible pathway for BoNT/A involvement.

Methods

A literature search was conducted in Pubmed, Ebscohost, Sciencedirect, and Google Scholar for articles published in English based on keywords : "HLA B27 Uveitis", "Periperhal Blood Mononuclear Cells", “Stimulation”, “Antigen” and “Botulinum Toxin A”. Because the rarity of similar cases, we also conduct literature search for immune response to botulinum neurotoxin A. Search terms included a combination of main keywords: "Peripheral Blood Mononuclear Cells", “Stimulation”, and “Botulinum Toxin A”.

The amino acid sequence of BoNT/A is searched obtained from The Universal Protein Resource while the sequence homology between BoNT/A and human protein were being searched from NCBI (National Center for Biotechnology Information) software BLAST (Basic Local Alignment Search Tool). The FASTA protein sequence of BoNT/A were obtained from UniProt. The algorithm chosen in the BLAST program was DELTA-BLAST, and expected threshold is 0.1.

Result

Eight articles are eligible for inclusion in this review. Eligible article published from 2002 until 2019 describing various peptide or antigen being used to stimulate inflammation from patients or laboratory animal with HLA B27. We classify these evidence as favouring autoinflammation mechanism or autoimmunity mechanism to understand better the role of antigen in HLA B27 disease. Unfortunately, none of these antigens includes BoNT/A.

Evidence favouring autoinflammation mechanism: Homodimer Formation Leading To TH17 Activation

Dendritic cells from HLA B27 positive individuals challenged with antigen express B27 homodimer in its cell surface. As demonstrated by Santos[11] et al, In the resting state, dendritic cells from HLA B27 individuals do not express homodimer, but in active state, homodimer is expressed within 48 hours after activation. It is important to note that homodimer formation is a trait that is specific to HLA B27 individuals and is associated with pathologic role.

HLA B27 homodimer might cause unopposed immune cell effector activation through its interaction with CD4+ IL-17+ T-helper cells (Th-17) that express KIR3DL2 receptor in its cell surface. As demonstrated by Bowness[12] et al Th-17 expressing KIR3DL2 receptor in its surface (KIR3DL2+ TH17) from HLAB27 are activated after stimulation using streptococcal antigen and APC expressing B27 dimer, marked by clonal expansion, increased IL-17 production, and evasion from apoptosis. This effect is less pronounced when T-cells is only stimulated using APC expressing B27 dimer alone.

TNF-α and IL-23 (both are cytokine involved in innate immune system) seems to play major role in HLA-B27 disease. Activated mononuclear cells in HLA B27 individuals showed increase in TNF-,α IL-6, IL-1α, and IL-1β and IL-23 gene expression as well as TNF and IL-6 protein. This is demonstrated in experiment by Van Tok[13] et al which stimulated mononuclear cells from HLA B27 transgenic mice, using inactivated *Mycobacterium tuberculosis* and comparing it mononuclear cells from HLA B7 transgenic rat and wild type. In later experiment , Van Tok [14] et al investigate the role of IL-23/IL-17 axis in the pathogenesis of HLA-B27 disease. HLA-B27 rats are immunised using inactivated *Mycobacterium tuberculosis* then treated with anti-IL23 in prophylactic or treatment settings. Cellular analysis showed that anti IL-23 can suppress production of IL-17A and IL-22 in prophylactic manner, but not therapeutic setting. In vivo

data showed that prophylactic treatment using anti-TNF or anti-IL23 after immunization with mycobacterium tuberculosis, halt progression of spondylarthritis or arthritis in rats.

Table 1. Evidence for antigen involvement in HLA B27 Associated Diseases

Evidence Supporting Autoinflammation Mechanism	Evidence Supporting Auto Autoimmunity Mechanism
Homodimer formation in sample dendritic cells from HLA B27 patient but not from healthy subject after stimulation with LPS within 48 hours. [11]	Immunising rats with HLA B27 peptide, C terminally elongated form HLA B27, cytokeratine, and retinal S-Ag cause uveitis in sample animal (44%, 66%, 27%, and 93% respectively). While streptococcal antigen and myelin bone protein did not cause uveitis. T Cells from rats immunised with B27 peptides showed cross reactivity with cytokeratine in-vitro[15]
Costimulation with SEB and APC expressing B27 homodimer showed Marked increase in KIR3DL2+ CD4+ TH17 and IL-17 production in stimulated T cells from patient with HLA-B27 arthritis. Additionally these cells also produce TNF-α and IFN-γ. [13]	Coimmunization of C terminally elongated form of HLA B27 peptide (B2702.60–84) with uveitogenic peptide PDSAg resulted in an aggravation of uveitis compared to coimmunization of PDSAg with peptide B2702PA or immunization with PDSAg alone Cocultivation of T-Celss with B2702.60–84 and retinal S-Ag (PDSAg, SAg.286), IRBP (PI536, PI731, PI1137) or a nonpathogenic streptococcal peptide (Strep) showed aggravation of T-cell proliferation compared with specific antigen alone or B27 peptide alone.[16]
Marked increased in TNF, IL-6, IL-1α, and IL-1β gene expression as well as TNF and IL-6 protein in supernatant of HLA B27 rat but not in wild type or B7 transgenic rat, indicating hyper-responsiveness of innate immune system, after immunization using inactivated mycobacterium[13] Prophylaxis using anti-TNF, but not in treatment manner, modify spondyloarthropathy in HLA B27 rats immunized with mycobacterium	HLA HLA B2705 can present the non-immunodominant epitope of EBV virus to CD8+ cells. This atypical presentation is not seen HLAB2709 that does not associate with diseases (proven by IFN-γ production).[17]
Inhibition of spondylarthritis and arthritis can be achieved using IL-23 inhibitor as prophylacxis but not in treatment manner.[14] Cellular analysis showed IL-17 and IL-22 are inhibited in transgenic rats white blood cells treated using IL-23 as prophylaxis but not as treatment.	Marked increase of IFN-γ in patients with HLA-B2705[18]

Evidence favouring autoimmunity mechanism:

Both HLA B27 peptide fragment and certain self-antigen can cause HLA-B27 related disease. The oldest study by Wildner[15] et al used HLA B27 peptide and cytokeratine to induce uveitis and arthritis (Table 3.4). Immunisation with HLA B27 peptide successfully induce uveitis, especially the C-elongated form of HLA B27 peptide, the result is comparable with retinal S-Ag. T-cells from immunised rats with B27 peptide showed cross reactivity with cytokeratine. Cytokeratine is expressed in ciliary body epithelium, retinal pigment epithelium, and dilator muscle of the iris. This crossreactivity might partly explain uveitogenic capacity of HLA B27 peptide, and

Further experiment by Moring[16] et al using rats showed that coimmunization of C terminally elongated form of HLA B27 peptide

(B2702.60-84) with S-retinal antigen resulted in aggravation of uveitis compared with immunisation with separate peptide alone. After demonstrating the capacity of HLA B27 peptide in worsening uveitis in vivo, the mechanism behind aggravation of uveitis is studied. *Different T-cell lines specific for retinal S-Ag, intra retinal binding protein (IRBP), and streptococcus antigen are stimulated with corresponding antigen alone or with B27 peptide.* It was found that stimulation with corresponding antigen and B27 peptide yields highest proliferation, showing the capacity of B27 peptide to aggravate inflammation

Another important feature HLA B27 is its capability of presenting non-immunodominant peptide from certain antigen, and causing clonal expansion of CD8+ T-cells. This specific feature, thought to play role in HLA B27 disease because non-clasical epitope presentation to CD8+ T-cells might activate self-reactive T-cells. This is demonstrated by Tedeschi[17] Using non-immunodominant peptide of EBV (EBNA3A), Tadeschi et al stimulate PBMC from HLA B2705 (subtype of HLA B27 that is prone to disease) and compared with PBMC HLA B2709 (subtype of HLA B27 that is not associated with disease), and showed robust IFN-γ production from HLA B2705 PBMC. Another study was done by Tedeschi[18] et al, showed the same result. Model protein study showed that computational analysis that left the A pocket partially

unfilled. Although partially filled, B27 can still present non-immunodominant peptide to CD8+ T Cells and elicit immune response.

Table 2. Immunologic Response to BoNT/A

Stimulant	Outcome
Inactivated botulinum neurotoxin A[9]	The patient group mounted significantly higher responses (mean SI, 2.64) (Pb10−20) than the controls (mean SI, 1.33). Seventy-two percent of treated patient samples gave positive response (SIN2.0) to BoNT/A. While, only 3% were positive in controls (Pb10−18)
Botulinum neurotoxin (in BOTOX formulation), Tetanus toxoid (TT), or combination of both	BOTOX did not induce any significant cell proliferation. TT activated the PBMC in six patients, who had also anti-tetanus toxin antibodies. Combination of TT and BOTOX showed additional cell proliferation Stimulation using BOTOX showed relative reduction of CD4 and CD8 naïve T-cells compared to CD4 and CD8 memory T-cells.
Inactivated botulinum neurotoxin A[19]	Seven proinflammatory gene were upregulated after RAW264.7 cells being stimulated with botulinum toxin Following exposure to BoNT/A, RAW264.7 cell expressed increased levels of TNF-α and NO in a dose-dependent manner. IL-6 was detected only when stimulated with more than 5 nM BoNT/A. Incubating RAW 2647 cells with polyclonal anti-TLR2, blocked the production of NO and TNF- α after BoNT/A stimulation. This effect was not seen in cells incubated with anti-TLR4, anti IgG1, or anti IgG2
31 Peptides from botulinum neurotoxin A heavy chain[19]	Majority of sample from patient treated with BOTOX recognized 3-9 peptides (shown by stimulation index >2.0), while 6 samples responded to less than 2. Only two sample recognized more than 10 peptides. No healty control subject showed any recognition towards the peptide
32 peptides from botulinum neurotoxin A light chain[20]	The majority of samples recognized 3–8 peptides, while 6 samples recognized larger numbers (9–13) of peptides. All of these samples showed SI >2.0 compared to 8 control subject that showed no SI > 2.0

Immunologic Response to BoNT/A, Homology to Human Protein, and Its Role In Triggering Uveitis

As antigen, BoNT/A stimulates both innate and adaptive immune response. The innate immune response is mediated through TLR-2 and induce production of TNF-α and nitrite-oxide. This finding is demonstrated by Kim[19] et al. Stimulation of following exposure to BoNT/A, macrophage cells **e**xpressed increased levels of TNFα and NO in a dose-dependent manner. When cells are treated with anti TLR-2, stimulation using BoNT/A did not cause any detectable production of TNFα and NO.

Adaptive immune response to BoNT/A is demonstrated by clonal expansion of T-Cells and production of neutralizing antibody. Clonal expansion of T-cells in response to BoNT/A is studied by Oshima,[10, 20, 21] et al and one by Vlata[22], et al discuss the adaptive immune response to botulinum neurotoxin A. All articles assess the response by measuring the proliferative index of the T-cells. Oshima et al also explore which peptides are the immunodominant peptide in either light[20] or heavy[21] chain of BoNT/A. Apparently, most studied patients can respond well to 3-13 peptides, with no peptides showing more dominance. Study by Vlata et al showed that stimulation by Botox did not cause cellular proliferation in PBMC, but additional cellular proliferation can be seen when PBMC is stimulated using tetanus toxoid and Botox. *However, immunologic response can still be observed in cells stimulated by Botox, that is the shift in T cells subset to memory T cells.*

Using Basic Local Alignment Search Tool (BLAST) provided by National Centre For Biotechnology Information (NCBI), we identify homologue human protein with BoNT/A.[23] We display two protein that is known to be expressed in the uveal tract (cytokeratine 8 isofrom 1 and 2) and has homology with BoNT/A in Figure 1. Interestingly, BoNT/A sequence that is predicted to be homologous with cytokeratin-8 is also used by Oshima[21] et al to stimulate T-cell proliferation. We also cross-reference with IEDB (Immune Epitope Database and Analysis Resource), and found that sequence 690-746 binds strongly with HLA B27.[24]

A

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BoNTA  690  VQTIDNALSKRNEK---WDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIIN  746
            ++T++N  +   +K    ++  K + T W    +  Q    R  M    E+     +  +
Sbjct  123  IKTLNNKFASFIDKVRFLEQQNKMLETKW--SLLQQQKTARSNMDNMFESYINNLRRQL-  179

BoNTA  747  YQYNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLN-QCSVSYLMNSMIPYGVKR  805
               +   +EK  +   + ++   + +  NK    INK    +    +   +  + +
Sbjct  180  ----ETLGQEKLKLEAELGNMQGLVEDFKNKYEDEINKRTEMENEFVLIKKDVDEAYMNK  235

BoNTA  806  LEDFDASLKDALLKYIYD---NRGTLIGQVDRLKDKVNNTLSTDIPFQLSKYVDNQRLLS  862
            +E     L+  L     +    R     ++  L+ ++++T S  +    S+ +D   +++
Sbjct  236  VE-----LESRLEGLTDEINFLRQLYEEEIRELQSQISDT-SVVLSMDNSRSLDMDSIIA  289

BoNTA  863  TFTEYIKNIIN------TSILNLRYESNHLIDLSRYASKINIGSKVNFDPIDKN  910
                  ++I N       S+  ++YE    L  L+          +K     +++N
Sbjct  290  EVKAQYEDIANRSRAEAESMYQIKYEE--LQSLAGKHGDDLRRTKTEISEMNRN  341
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B

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BoNTA  690  VQTIDNALSKRNEK---WDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIIN  746
            ++T++N  +   +K    ++  K + T W   +  Q    R  M    E+      +  +
Sbjct  95   IKTLNNKFASFIDKVRFLEQQNKMLETKW--SLLQQQKTARSNMDNMFESYINNLRRQL-  151

BoNTA  747  YQYNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLN-QCSVSYLMNSMIPYGVKR  805
            +   +EK  +   + ++   + +  NK    INK    +     +   +    + +
Sbjct  152  ----ETLGQEKLKLEAELGNMQGLVEDFKNKYEDEINKRTEMENEFVLIKKDVDEAYMNK  207

BoNTA  806  LEDFDASLKDALLKYIYD---NRGTLIGQVDRLKDKVNNTLSTDIPFQLSKYVDNQRLLS  862
            +E     L+  L     +    R     ++  L+ ++++T S  +    S+ +D   +++
Sbjct  208  VE-----LESRLEGLTDEINFLRQLYEEEIRELQSQISDT-SVVLSMDNSRSLDMDSIIA  261

BoNTA  863  IFTEYIKNIIN------TSILNLRYESNHLIDLSRYASKINIGSKVNFDPIDKN  910
                ++I N       S+  ++YE   L  L+          +K     +++N
Sbjct  262  EVKAQYEDIANRSRAEAESMYQIKYEE--LQSLAGKHGDDLRRTKTEISEMNRN  313
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Figure 1. Alignment between BoNTA amino acid sequence with cytokeratine 8 isoform 1 (A) and 2 (B). The top line represents protein sequence of BoNT/A, the bottom line is the respective human protein. The middle line reports the quality of the match. Letter is identical match, and (+) sign is positive match (considering it as conservative substitution). The percentage amino acid identity of both protein with BoNT/A is 15%.

We hypothesised that BoNT/A triggers uveitis in HLA B27 individual through aberrant dendritic cells. Data from Santos[11] et al showed that antigenic activation of dendritic cells might lead to B27 homodimer formation and Bownes[12] et al demonstrate that B27 homodimer might interact with TH-17 expressing KIR3DL2. This interaction activate TH-17, aggravating its proliferation and IL-17 formation. Dendritic cell respose to BoNT/A stimulation is mediated by TLR-2, leading to its activation. Because innate immune system responds to various antigenic stimuli in similar pattern, homodimer will be formed upon stimulation.

At the same time, classical antigen presentation might still occur. Antigen presented by dendritic cells of HLA B27 individuals might present immunodominant or non-immunodominant epitope. This flexibility of antigenic presentation, as demonstrated by Tedeschi[17, 18] et al, might activate self-reactive CD8+ T-cells. BoNT/A is an antigen with some homology with cytokeratin, we propose that HLA B27 present the homologous portion of BoNT/A protein to autoreactive CD8+ , activating it, leading to tissue destruction. The pathway leading to tissue destruction is summarised in Figure 2.

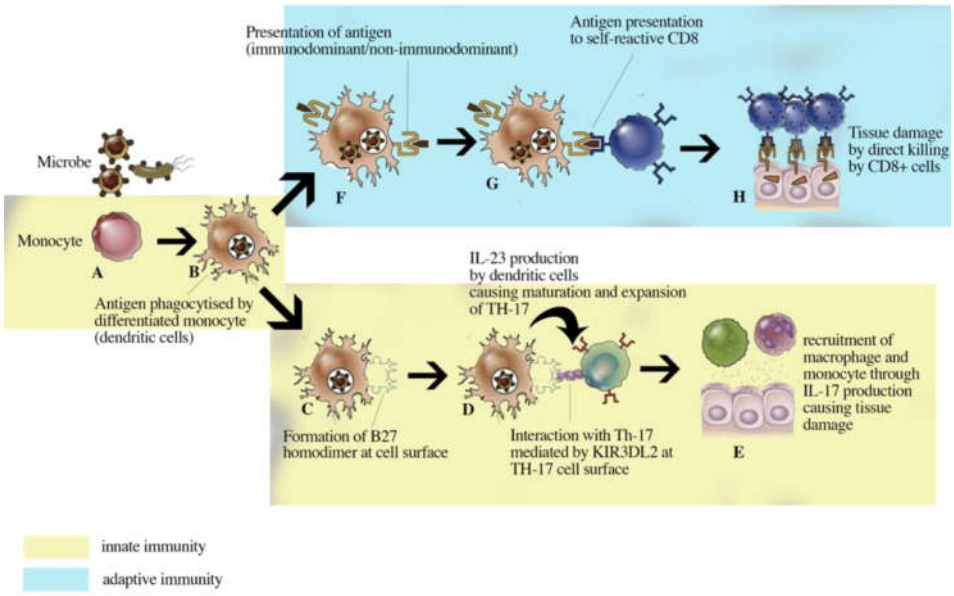

Figure 2 Proposed common immunopathology pathway in HLA B27 disease shared by innate and adaptive immune response, and where BoNT/A exert its role. A) Microbial insult will result in activation of innate immune system B) phagocytosis by macrophage, among them dendritic cells. C) Activation of dendritic cells through TLR pathway will lead to formation of HLA B27 homodimer. BoNT/A migh act similarly with other antigen only differs in sub-type of TLR activated D) dendritic cells with homodimer interacts with Th-17 activating it ultimately leading to tissue destruction by recruitment of macrophage and monocyte to target tissue. At the same time, F) classic antigen presentation by dendritic cells to CD8+ T Cells mark the beginning of adaptive immune response. G) Flexibility of antigen epitope presentation by dendritic cells might activate self reactive CD8+ T cells causing H) clonal expansion and tissue destruction. In this case HLA B27 might present portion of BoNT/A that is homologous to self protein to CD8+ self reactive T-cells.

Conclusion

Our literature review establish evidence linking the introduction of BoNT/A to HLA B27 patient induces uveitis. Although there is no single literature use BoNT/A to stimulate PBMC from HLA B27 patients, we can still establish two pathways in which BoNT/A might involve in HLA B27 disease. We found that BoNT/A can elicit both innate and adaptive immune response, which in physiological state is protective; however deviant immune response is seen in HLA B27 individual, and stimulation with BoNT/A can upregulate the expression of B27 homodimer in cell surface and atypical antigen presentation.

However there are still several questions that need to be addressed through experiment such as : does BoNT/A cause proliferation of pathologic T-cell lines, does homologue portion of BoNT/A presented by APC in HLA B27 individuals. Experiment by stimulating PBMC in vitro from our patients compared with other HLA B27 patients and non-HLA B27 anterior uveitis patient, patient with prior BoNT/A injection and normal population using flowcytometry might help understand the role of BoNT/A in triggering uveitis. Answering these question might help us better in answering the role of BoNT/A, and antigen, as trigger of uveitis in human.

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