Epsilon toxin, Multiple Sclerosis and Vaccine Candidate (Y30A-Y196A-A168F)

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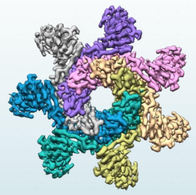


One Health Ventures

Executive Summary

- **Epsilon toxin(ETX) is a lethal toxin known to cause enterotoxaemia in sheep**
- **ETX now linked to Multiple Sclerosis (MS) in humans**
- **Myelin and Lymphocyte (MAL) is identified as key binding site for ETX**
- **MAL is expressed in all cell lines damaged by MS**
- **There is no human vaccine against ETX**

One Health Ventures Limited (OHV) has developed a proprietary, recombinant, genetic toxoid vaccine candidate against epsilon toxin (ETX), which can be cultured in E.coli, as a treatment for both animals and humans. OHV is seeking a commercial partner(s) either to licence or enter into a joint venture(s) in order to exploit its proprietary vaccine against ETX in both animal and human medicine worldwide.

Background

ETX is the third most lethal toxin known to humans and is classified as a category B bio-terrorism agent by the U.S. Government's Centers for Disease Control and Prevention. ETX is a heptameric, pore-forming toxin (see logo) produced by a plasmid in certain strains of Clostridium perfringens (types B and D). ETX is the cause of enterotoxaemia (also known as "pulpy kidney" disease) in livestock, mainly sheep, goats and cattle. The plasmid initially produces ETX as an inactive "prototoxin" in the gut, which is then activated by an enzyme (typically trypsin or chymotrypsin), which is prevalent in the gut. Once in its active state the toxin crosses the intestine wall and thereby enters the bloodstream where it binds to cells expressing Myelin and Lymphocyte Protein (MAL). Sheep and other animals can die rapidly (within hours) from an infection.

Over the past 7 years, ETX has been increasingly implicated as the trigger for Multiple Sclerosis (MS) in humans, which affects about 2.5 million people worldwide and for which there is currently no cure. Evidence includes finding significant levels of ETX, antibodies to ETX and culturing the relevant bacterial strains from MS patients while observing much lower frequencies in healthy controls. There is also currently no human vaccine against ETX.

The only vaccines currently available against ETX are for livestock and are based on formaldehyde-treated C. perfringens, are impure and produce low or variable yields. In addition, immunogenicity of the ETX toxoid is low requiring frequent booster doses. **The new genetic toxoid vaccine candidate overcomes all these problems.**

What is Multiple Sclerosis?

Multiple Sclerosis (MS) is defined as a chronic condition in humans that affects the brain and spinal cord (i.e. the central nervous system (CNS)). In MS, the oligodentrocytes are damaged (demyelinated) and this causes a range of symptoms including problems with vision (such as Optic Neuritis), muscle weakness affecting arm or leg movement and trouble with sensation or balance. Oligodentrocytes produce the insulating coating (myelin) that protects the nerve cells (axons). MS is typically identified and characterized by lesions in the brain.

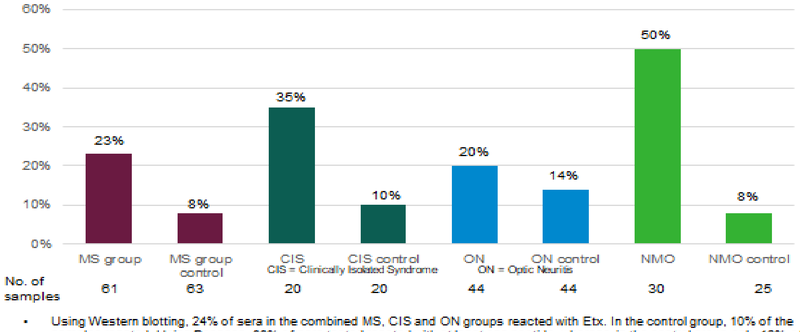
MS takes several forms. The most common form of MS is Relapsing Remitting MS (RRMS), with which new symptoms occur in isolated attacks. Less common, but more severe, are the progressive forms of Secondary Progressive MS (SPMS) and Primary Progressive MS (PPMS), in which MS attacks build up over a shorter time. In RRMS, attacks may be months or years apart and symptoms may disappear completely.

Table 1 to show Commonality between Epsilon Toxin and Multiple Sclerosis

Characteristics	Epsilon Toxin	Multiple Sclerosis	Cells Expressing MAL
Damages and Crosses Blood Brain Barrier	✓	✓	✓
Damages Oligodentrocytes	✓	✓	✓
Damages Blood Retina Barrier	✓	✓	✓
Damages Lymphocytes (T Cells not B Cells)	✓	✓	✓
Damages Human Red Blood Cells	✓	✓	✓
Attacks Myelin	✓	✓	N/A
Increases Levels of Ceramide: 16 and 24	✓	✓	N/A
Causes Accumulation of Neurofilaments	✓	✓	N/A
Elevates Glutamate Levels in Serum	✓	✓	N/A
Generates Excess Sulfatide	✓	✓	N/A

Table 2

Comparison between NMO patients and MS patients (testing positive for antibodies to ETX)



- Using Western blotting, 24% of sera in the combined MS, CIS and ON groups reacted with Etx. In the control group, 10% of the samples reacted. Using Pepscan, 33% of sera tested reacted with at least one peptide, whereas in the control group only 16% of sera reacted. Out of 61 samples, 21 (43%) were positive to one or other testing methodology
- NMO patients on limited sample size showed double the rate (50%) of reactivity to ETX antibodies compared to the average for MS/CIS/ON patients (24%)

Evidence of Clostridium perfringens epsilon toxin associated with multiple sclerosis.
Sariqa Wagley, Monika Bokori-Brown, Helen Morcrette, more. Published: 21 April 2018.
https://journals.sagepub.com/doi/full/10.1177/1352458518767327

Continued overleaf

MS also involves an immune-mediated process in which an abnormal response (possibly caused by memory B cell lymphocytes) of the body's immune system is directed against myelin in the CNS, which is why MS is frequently referred to as an "auto-immune" disease. It is also well-known that MS patients frequently experience "fatigue". Now that research has been published (see below), which links the expression of MAL to RBCs, then this could explain the cause of the fatigue.

What Relevance does MAL have to ETX and MS?

There is increasing evidence that indicates cells expressing MAL are the key receptors for ETX i.e. are the main binding sites. It has been widely shown that ETX rapidly damages the Blood Brain Barrier (BBB) of mice and sheep before damaging Oligodentrocytes. MAL in mice and sheep is 100x more reactive than human MAL. It is also known that human MAL, unlike in mice and sheep, is expressed in a significant proportion of human CD4s and to a lesser extent CD8 lymphocytes. K Rumah has also very recently found that an isomer of MAL, unusually, is expressed in human RBCs only – but not in sheep, goat, guinea pig, monkeys (rhesus macaques) or cows. He has also identified that in humans the impact of ETX is enhanced by the presence of CD4s expressing MAL (a different isomer which is more reactive than the MAL in RBCs).

It is widely accepted that the following cell lines - blood brain barrier, blood retina barrier, oligodentrocytes, lymphocytes and red blood cells – are all specifically damaged as part of MS. It is also known that these cell lines all express human MAL and all are capable of being specifically damaged by ETX (Table 1).

Ironically, it may transpire that humans are "luckier" than other animals in that their RBCs and CD4s react with ETX before it gets to the more sensitive areas such as the BBB or brain. It is also known that human MAL is much less reactive to ETX than sheep or mouse MAL. These factors may help to explain why humans are able to tolerate ETX exposure better than sheep, mice or rats.

Therefore it is hypothesised that the effect of ETX toxicity is significantly reduced in humans compared with sheep, mice and rats because human MAL is less reactive and because the RBCs and CD4+ lymphocytes act as a buffer or "sponge" to mop up a significant proportion of the toxin and thereby reduce the impact of the toxin on the human brain compared to that inflicted on the brains of mice and sheep.

Summary of the Evidence Linking ETX to MS

Western blotting analysis of serum from 125 MS patients and 125 controls was carried out to identify if more MS patients test positive for antibodies to ETX compared to controls. The resultant paper found that, typically, 23% to 35% of MS patients tested positive compared with 8% to 14% of controls (Table 2). Pepscan analysis was also carried out on samples from 75 of the 250 people referred to above (57% with some form of MS diagnosis and 43% without). 33% of those people with MS reacted with at least one peptide of ETX (and most recognised several peptides), compared to 16% among the control group. In summary, the results found that of those who were tested under two separate methods (Western blotting and Pepscan), a total of 43% of MS patients and 16% of controls were found to be positive by at least one assay. These are significant results in the search for the elusive trigger of MS (Table 5).

More recently, as yet unpublished results from a pilot study of 30 patients with neuromyelitis optica (NMO), which in the past has been implicated as a "sister disease" to MS as there are many commonalities, indicate that these patients have an even higher incidence of antibodies to ETX. The incidence of antibodies to ETX found in these patients was 50% compared with 8% for controls (Table 2).

Jennifer Linden has presented a poster at ECTRIMS 2018, in which she managed to detect ETX on CD4+ cells in 33% of MS patients compared with only 8% for controls (Table 3). At the same conference, S Haigh was able to show that C. perfringens (type B or D) could be cultured from 21% of MS patients compared with 0% from controls (Table 4).

The Vaccine Candidate (Y30A-Y196A-A168F)

OHV, in association with Professor Rick Titball and Exeter University in the UK, has developed a "genetic toxoid" of epsilon toxin (Y30A-Y196A-A168F) (Figure 1). Unlike the existing animal vaccine methodology, this means that the protein can be cultured in E. coli and does not require any processing to render it safe to use a vaccine. Y30A-Y196A-A168F is safe when tested towards different cultured cells, and safe and efficacious when tested in mice, rabbits and sheep.

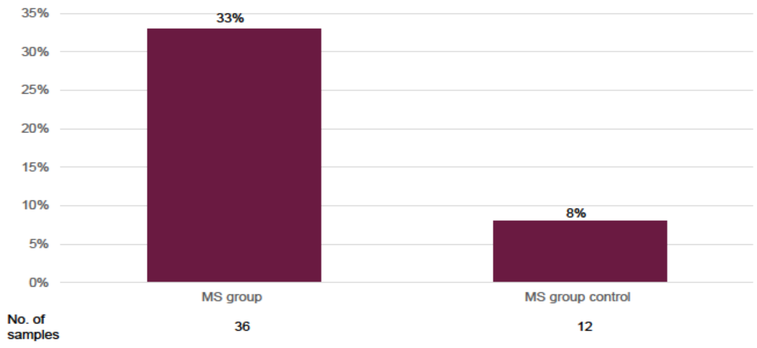
The immunisation of sheep with Y30A-Y196A-A168F and Montanide ISA 61VG adjuvant results in very high levels of neutralising antibodies which have been found to persist for at least one year. Montanide is not accepted as an adjuvant for human use so a different choice of adjuvant will be needed. Immunisation of sheep with a single dose of vaccine is sufficient to generate high levels of neutralising antibodies. The vaccine candidate has also been shown to have negligible reactivity with human erythrocytes (red blood cells).

References

1. Kareem Rashid Rumah,Jennifer Linden,Vincent A. Fischetti,Timothy Vartanian (2013) Isolation of Clostridium perfringens Type B in an Individual at First Clinical Presentation of Multiple Sclerosis Provides Clues for Environmental Triggers of the Disease (PlosOne)
2. Kareem Rashid Rumah ,Yinghua Ma ,Jennifer R. Linden ,Myat Lin Oo,Josef Anrather,Nicole Schaeren-Wiemers,Miguel A. Alonso,Vincent A. Fischetti,Mark S. McClain,Timothy Vartanian (2015) The Myelin and Lymphocyte Protein MAL Is Required for Binding and Activity of Clostridium perfringens ε-Toxin (Plos Pathogens)
3. Sariqa Wagley, Monika Bokori-Brown, Helen Morcrette, Andrea Malaspina, Caroline D'Arcy, Sharmilee Gnanapavan, Nicholas Lewis, Michel R Popoff, Dominika Raciborska, Richard Nicholas, Ben Turner, Richard W Titball (2018) Evidence of Clostridium perfringens epsilon toxin associated with multiple sclerosis (Multiple Sclerosis Journal)
4. Helen Morcrette, Monika Bokori-Brown, Stephanie Ong, Leo Bennett, Brendan W. Wren, Nick Lewis & Richard W. Titball (2019) Clostridium perfringens epsilon toxin vaccine candidate lacking toxicity to cells expressing myelin and lymphocyte protein (NPJ Vaccines)
5. Rashid Rumah, Olawale E. Eleso & Vincent A. Fischetti (2019) Human blood exposure to Clostridium perfringens epsilon toxin may shed light on erythrocyte fragility during active multiple sclerosis (Biorxiv)

Table 3

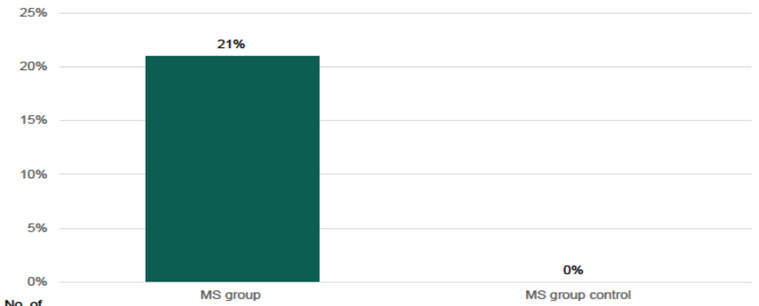
ECTRIMS Poster (October 2018) by Jennifer Linden on epsilon toxin and CD4s and CD8s in MS patients entitled: *"Analysis of CD4+ cells reveal increased exposure of multiple sclerosis patients to clostridium perfringens epsilon toxin"*



Epsilon toxin detected on CD4+ cells of serum from various MS patients and controls. J Linden identified toxin in 12 of 36 MS patients (33%) compared with 1 in 12 controls (8%).

Table 4

ECTRIMS Poster by S Haigh showing C perfringens grown from faecal samples of MS patients but not controls entitled: *"Intestinal Colonization by Epsilon Toxin-producing Clostridium perfringens Strains is Associated with Multiple Sclerosis"*



C. Perfringens (type B or D) cultured from 6 of 28 faecal samples from MS patients and zero bacteria were cultured from controls

Table 5

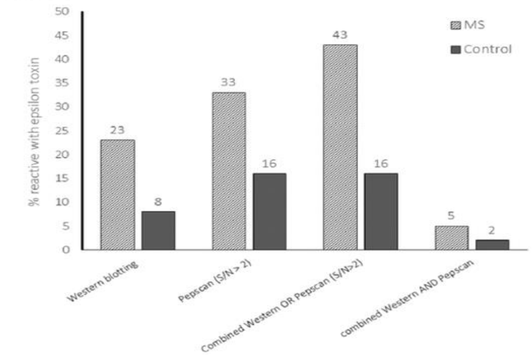


Figure 1