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Updates on tetanus toxin: a fundamental approach

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ABSTRACT

***Clostridium tetani* is an anaerobic bacterium that produces second most poisonous protein toxins than any other bacteria. Tetanus in animals is sporadic in nature but difficult to combat even by using antibiotics and antiserum. It is crucial to understand the fundamental mechanisms and signals that control toxin production for advance research and medicinal uses. This review was intended for better understanding the basic patho-physiology of tetanus and neurotoxins (TeNT) among the audience of related field.**

Keywords

Soil borne disease, Spore, Neuron, Toxins

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INTRODUCTION

Tetanus is an acute, often fatal disease caused by the exotoxin of *Clostridium tetani,* characterized by generalized muscle rigidity and autonomic instability (Freshwater-Turner et al., 2007). *C. tetani* is a motile, spore-forming and obligate anaerobic bacterium with a natural habitat of soil but can also be isolated from feces of domestic animals and humans (Wilkins et al., 1988; Afshar et al., 2011). Tetanus is considered as one of the most dramatic and cosmopolitan diseases of humans and vertebrate animals for over 24 centuries (Bruggemann et al., 2003). Inoculation with *C. tetani* spores typically occurs through a contaminated wound but in 20% of cases the sources of infection may be unknown (Ogunrin, 2009). As an obligate anaerobe, the bacillus cannot grow in healthy oxygenated tissue, thus wounds are usually associated with co-infection, necrotic tissue, a foreign body or localized ischaemia (Cook et al., 2001; Schloss et al., 2011).

The incubation period of tetanus varies from eight days to several months (Vandelaer et al., 2003; Brauner et al., 2002) based on the location of injury site from the central nervous system (CNS). Severity of symptoms is incubation period dependent: shorter the incubation period, the more severe the symptoms and vice versa (Farrar et al., 2000; Brook, 2008; Afshar et al., 2011). Under anaerobic conditions the spores germinate and the bacteria produce tetanus neurotoxin (TeNT), which is released by bacterial autolysis and enters the body fluids. Followed by reaching the main peripheral targets of this toxin that is presynaptic membrane of motoneurons nerve terminals (Rossetto et al., 2014). There is also evidence that TeNT may bind to sensory and adrenergic terminals (Habermann and Dreyer, 1986). In immunocompromised subjects TeNT can block inhibitory neurons causing hyperreflexia, muscle hypertonia and muscle spasms; sympathetic hyperexcitability and increased circulating catecholamine levels (Gomes et al., 2011). Tetanus is designated as notifiable disease at the national level in the countries having disease burden (CDC, 2010). It is an occupational zoonotic disease and veterinarians, physicians and other zoo staffs coming in contact are in risk of tetanus zoonosis (Chethan-Kumar et al., 2013).

C. TETANI STRAINS

The *C. tetani* species contains toxigenic and non-toxigenic strains and is similar culturally and biochemically to *C. cochlearium* and *C. tetanomorphum*, but it can be distinguished from the two latter species by DNA comparison (16S rDNA) (Nakamura et al., 1979; Wilde et al., 1989; Kalia et al., 2011). Comparative genomic analysis is possible using partial genome sequence, and phylogenesis of a few conserved

proteins involved in cellular processes and metabolism. A genome sequence is available for only one *C. tetani* strain (Bruggemann et al., 2003; Alam et al., 2010).

FACTORS RELATED TO BACTERIAL COLONIZATION

The spores are noninvasive and require a skin break for germination. Hosts having wound that is contaminated by soil and with low oxygen tension are optimal locations for *C. tetani* under optimum temperature at 37°C in vivo (Ernst et al., 1997). To defeat oxygen tension a few identified systems such as superoxide dismutases, peroxidases and heme oxygenase (*hemeT* gene) are probably responsible for protection (Brüggemann et al., 2004). Bacterial collagenases also play a crucial role in host colonization (Eckhard et al., 2014). Among all animal species, horses, goats, sheep, monkeys (*Macacus rhesus*) and cattle, which are sensitive to the toxin of *C. tetani*, but dogs are relatively resistant, and cats are more resistant (Shumacker et al., 1939; De Risio and Gelati, 2003). In reality, the resistance of avian species to tetanus is due to a mutation at the cleavage site for VAMP (synaptobrevin) (Hamza and Abdellah, 2011). Thus, host is an important factor to set an infection.

GERMINATION OF SPORE INTO HOST

To cause disease spores must return to active vegetative form. Bacterial spore germination is induced when specific environmental cues, termed germinants, are sensed by specific germinant receptors (GRs) (Olguín-Araneda et al., 2014). Upon binding of the germinant to the GR, a series of irreversible biophysical and biochemical reactions are triggered which lead to the degradation of the spore's peptidoglycan (PG) cortex, allowing the rehydration of the spore core and resumption of metabolism (Paredes-Sabja et al., 2011). Notably, although spores germinate normally under anaerobic conditions (Sorg et al., 2008; Paredes-Sabja et al., 2008), presence of oxygen hampers subsequent development and growth of the nascent vegetative cell (Plowman et al., 2002).

MOLECULAR BASIS OF TOXINS PRODUCTION

The *C. tetani* genome is composed of a chromosome that contains 2,799,250 bp and a plasmid, pE88 that contains 74,082 bp (Bruggemann et al., 2003). The neurotoxin genes are encoded in the plasmid (Marvaud et al., 2000). Actin like protein (Alp12) is suggested a dynamically unstable force-generating motor involved in segregating the pE88 for TeNT (Popp et al., 2012). The *C. tetani* locus (Dupuy et al., 2006) contains the toxin gene *tetX* and the accessory regulatory gene *tetR* (Marvaud et al., 2000; Carter et al., 2013). The *tetR* found upstream of the *tetX* gene (Marvaud et al., 1998a; Marvaud et al., 1998b). In order to protein regulators, bacteria utilize another class of regulatory molecule known as small regulatory RNAs know as sRNA that have been identified in *C. tetani* (Chen et al., 2011). The sRNA can vary in length from 50 to 300 nucleotides and act either in *cis* or in *trans* (Storz et al., 2011). Most of the sRNAs interact with mRNA targets through an antisense mechanism, and can alter transcription, translation and/or mRNA stability of target genes for TeNT production (Lalaouna et al., 2013). Thus, interaction of sRNAs influences a wide range of cellular processes including toxins production and virulence processes (Ternan, 2013), and their role being subjected to change in response to stress (Venkataramanan et al., 2013).

TOXINS BIOLOGY

The tetanus bacillus secretes two toxins: tetanospasmin and tetanolysin (Cook et al., 2001). Tetanolysin is capable of locally damaging otherwise viable tissue surrounding the infection and optimizing the conditions for bacterial multiplication (Pinder, 1997). Tetanus produces tetanospasmin. This toxin may constitute >5% of the weight of the microorganism (Mellanby, 1968). Tetanus toxin gene is encoded on a 75-kb plasmid, and synthesised as a single polypeptide with a molecular weight (MW) of 150,000. The complete amino acid sequence of the toxin is known from gene cloning (Finn et al., 1984; Eisel et al., 1986; Fairweather et al., 1986). Neurotoxin share a common structure composed of a heavy (Hc; 100 kDa) and a light (Lc; 50 kDa) chain linked by a disulphide bond (Herreros et al., 1999). The amino-terminal domain H_N (fraction of Hc domain) is responsible for translocating the L_C across the plasma membrane, whereas the carboxyl-terminal domain H_C is responsible for the binding of TeNT to gangliosides on neurons (Rummel, 2003). These three functional domains are structurally distinct, and are arranged in a linear fashion, such that there is no contact between the L_C and H_C domains (Lacy and Stevens, 1999; Turton et al., 2002).

TRANSMISSION OF TETANOSPASMIN FROM INFECTION SITE

TeNT spreads from the infected site by diffusing into the adjacent muscle tissue by being transported via lymphatic system or by nerves passages (Baldassi, 2005). TeNT enters the blood from the lymphatic

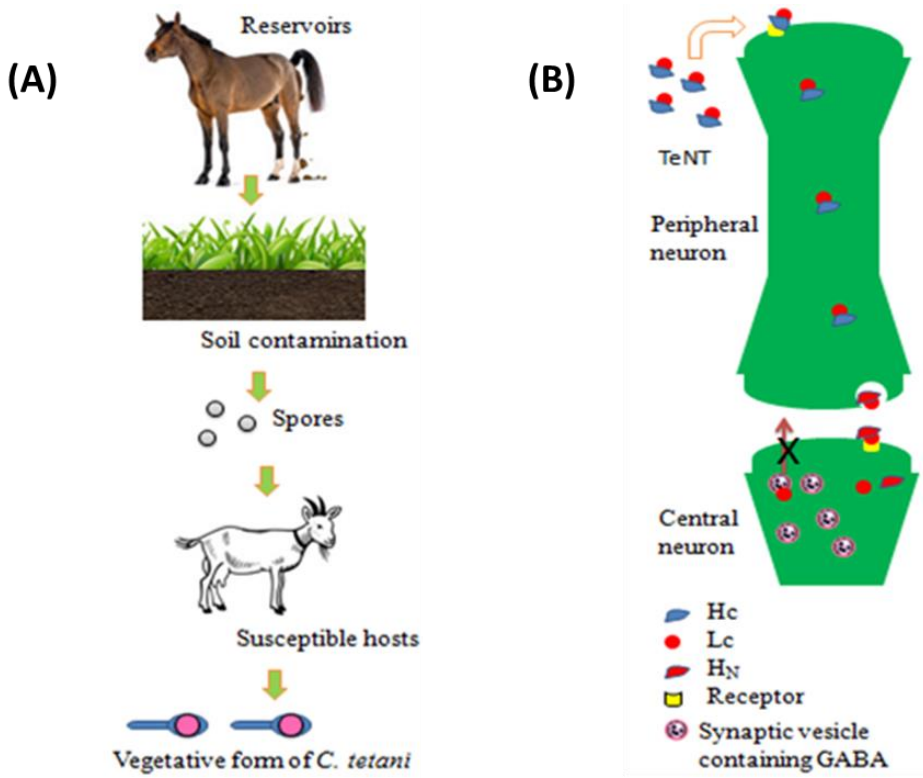

Figure 1: (A) Common pathway of tetanus infection in susceptible host. **(B)** TeNT molecules are transported along the axon of the lower motor neuron to the central neuron of CNS, where the catalytic Lc chain is transcytosed into inhibitory interneurons and blockade of synaptic vesicle to release GABA, resulting in uninterrupted excitatory impulses and signs of tetani.

system, attaches to a receptor on the nerve ending, and a fragment of the bound toxin is taken into the nerve cell and passes on to the CNS by retrograde movement through the nerve axons (Veronesi and Focaccia, 1981; Rossetto et al., 2014). The TeNT is 2,000 times more toxic at central inhibitory nerves than at peripheral synapses (Morton and Meunier-powell, 1997).

CELL ENTRY STRATEGY

Bindings to cell surface receptor: Tetanus toxin binds to the adjacent motor neuronal membranes of terminals and cell bodies. The heavy chain (Hc) plays a major role in specific binding to the neuron (Binz and Rummel, 2009). Tetanus toxin binds specifically to polysialogangliosides (GD1b, and GT1b), as well as cell surface proteins (von Bartheld, 2004).

Internalization: Debates are exists about the Internalization process of TeNT, either the coated-pit pathway (Parton et al., 1987) or in noncoated pits (Herreros et al., 1999). One receptor for tetanus toxin was identified as the Thy1 protein, a common GPI-anchored protein on surface membranes of projection neurons. Additional receptors for tetanus toxin may include the p75 neurotrophin receptor (Butowt and von Bartheld, 2003). Both the Thy1 and p75 proteins are preferentially associated with lipid rafts and binding of surface antigen (Sheets et al., 1997; Bilderback et al., 1999; Fewou et al., 2014). Endocytosis of tetanus toxin into presynaptic motor terminals requires presynaptic electrical activity, but not postsynaptic stimulation (Miana-Mena et al., 2002).

Axonal transport: Channel formation is enhanced by receptor binding and dependent on acidic lipids that are modulated by the membrane environment (Burns and Baldwin, 2014). It is then moved from the peripheral to the CNS by retrograde axonal transport (Schiavo et al., 2000) as well as anterograde axonal transport (Manning et al., 1990).

In central neurons: The entire toxin molecule is internalized into presynaptic cells and, in a process requiring the H_N fragment, the Lc is released from the endosome. The metalloprotease activity of the tetanus neurotoxin (TeNT) light chain cleaves the neuron-specific soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein called vesicle-associated membrane protein 2 (VAMP2, SV2 or synaptobrevin 2) (Schiavo et al., 1992; Yeh et al., 2010;

Blum et al., 2012). Synaptobrevin is an integral membrane component of synaptic vesicles and is essential for the fusion of synaptic vesicles with the presynaptic membrane (Li et al., 1994). Cleavage by tetanus toxin Lc prevents release of their contents, the inhibitory neurotransmitter γ-aminobutyric acid (GABA), into the synaptic cleft (Bleck, 1986; Salinas et al., 2010). The α motor neurons are under no inhibitory control, and undergo sustained excitatory discharge, causing the characteristic motor spasms of tetanus (Ataro et al., 2011) (**Figure 2**). The toxin exerts its effects on the spinal cord, the brain stem, peripheral nerves, at neuromuscular junctions, and directly on muscles (Farrar et al., 2000) mediated by bindings with the synaptic vesicle binding protein SV2A and SV2B (Yeh et al., 2010) (**Figure 1**).

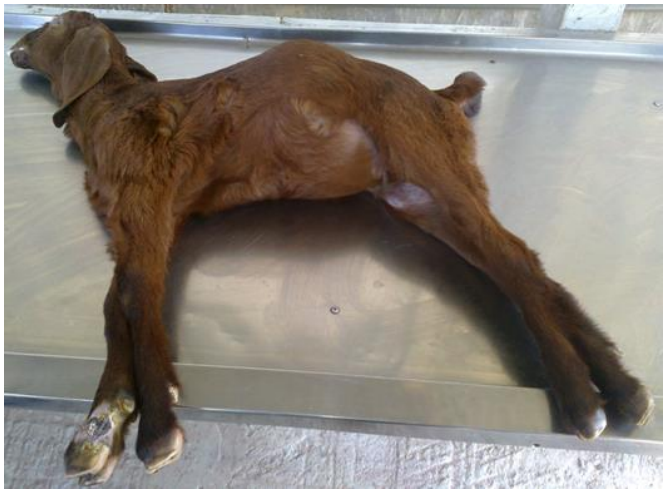

Figure 2: A five months old Jamunapari goat is suffering from tetanus with typical signs: locked jaw and stiffness of the body after an accidental wound (right forelimb) and use of unhygienic cloth as a bandage material in injured part by the owner himself. The signs appeared nine days post-accident.

FATE OF TOXINS

The toxin has a half-life of 5-6 days. Both the heavy and the light chains of tetanus toxin are degrading at similar rates (Habig et al., 1986). Neuronal binding of toxin is irreversible thus recovery requires the growth of new nerve terminals, which explains the prolonged course (6-8 weeks) of tetanus (Bleck, 1987; Thwaites et al., 2014). Therefore, antitoxin should be given as soon as possible for management (Thwaites, 2014). In the developed world, this is usually human-origin (tetanus immune globulin or human immunoglobulin) but equine forms are also available (Kabura et al., 2006).

CONCLUSION

Despite an ever-increasing amount of knowledge related to *C. tetani* and neurotoxin, the problem is still unresolved. New developments in our understanding of the tetanus toxin and the *C. tetani* organism can help for better treatments, prevention and control. Public awareness is unbeatable and need to be emphasized among the new generation practitioners.

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