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

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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 (Brüggemann 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) (Olgui-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 (Brüggemann 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). Tetanospasmin 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 HN (fraction of Hc domain) is responsible for translocating the Lc across the plasma membrane, whereas the carboxyl-terminal domain Hc 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 Lc and Hc 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.

**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 HN 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).

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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