affected the cellular barrier also indicating the presence of other bioactive compounds.

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**Study on the usefulness and limitations of a cytotoxicity bio-assay using KB cells to detect lipophilic toxins in shellfish matrices**

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Mouse bioassays have been widely used to detect shellfish toxins. Due to major drawbacks, a general trend exists to move away from this assay. Thus, the development of alternative methods becomes necessary for the detection of emerging toxins. Previous studies demonstrated potential of cell-based assays for detecting lipophilic toxins. The present work aimed at complementing these studies through critical assessment of a cytotoxicity assay based on KB cells. Cell-based assays have no ethical concern, and present different advantages: ease of use, low requirement of laboratory facilities, low extract consumption and possibility of high-throughput. Moreover, KB cells are robust, with a low sensitivity to variations of culture conditions. Studies were carried out at different levels of matrix: pure toxins, algal and mussel extracts. KB cells were capable of discrimination between toxic and non-toxic micro-algae, with kinetic studies suggesting a minimum exposure of 48 h, even for pure toxins. Matrix effects of mussels were evaluated with crude and purified extracts of mussel hepatopancreas. Maximum concentrations of matrix not interfering with the assay were determined for different levels of purification. Even when extracts were partially purified, matrix effects observed represent a major drawback of this assay. When respecting maximum matrix concentration, the LOD for OA was around the regulatory limit while the LOD for AZA1 was 100-fold below the regulatory limit. Low sample consumption makes the use of this test attractive for the search of unknown toxins. However, matrix effects in shellfish and large difference in LODs between toxins are significant obstacles for regular use in surveillance programmes.

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**In vivo and in vitro studies with a novel human monoclonal antibody against tetanus neurotoxin**

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Tetanus neurotoxin (TeNT) synthesized by the anaerobic *Clostridium tetani* is one of the most deadly bacterial toxins known to humans, and is responsible for the severe neurological illness known as tetanus characterized by generalized muscle spasms and rigid paralysis. TeNT is known to enter motor nerve terminals of the neuromuscular junction and is retrogradely transported along the motoneuron axons to the cell body. TeNT is transcytosed into adjacent inhibitory interneurons where it blocks the release of inhibitory neuro-transmitters (i.e., gamma-aminobutyric acid and glycine), leading to spastic paralysis. Vaccination is the best way to prevent tetanus disease, but its protection does not last forever. Adults should get a tetanus shot, or booster, every 10 years. In the present work we have studied the efficacy of fully human recombinant monoclonal antibodies (Mabs) directed to TeNT, and obtained from B-cells of a recently immunized blood donor, using “single cell PCR” method. This approach entails the isolation of B-cells by a cell sorter coupled to a cytometer using a B-cell selective marker, anti-CD19 associated to a B-cell specific marker, C-ter fragment of TeNT-FITC-labeled. RT-PCR on single cell allowed the conversion of total RNA to cDNA, which was amplified using specific primers to select variable regions of the heavy and light chain of IgG. These variables regions were cloned into an expression vector containing the corresponding constant part of human IgG1. The expression vectors were then transiently transfected into CHO cells before purification of the expressed Mabs. Several new antitetanus Mabs were characterized in order to demonstrate their protective quality as compared to that of the therapeutic human polyclonal antibodies, Gammatumanos (LFB). Mice were injected with different doses of antibodies combined to lethal doses of TeNT. One of the tested Mabs exhibited a remarkable efficacy at nano-gram concentrations. In addition, we have tested the capacity of this human Mab to inhibit the binding and/or translocation of TeNT within motor nerve terminals. For this, we have used a fusion protein composed of the atoxic C-terminal fragment of TeNT coupled to the fluorescent probe Cy3 and isolated mouse neuromuscular preparations with or without the presence of antibodies. Confocal microscopy revealed that the fusion protein rapidly clustered inside motor nerve terminals of the neuromuscular junction under control conditions, and this internalization was markedly reduced when the novel effective human Mab was used. Comparison with Gammatumanos was also performed. Our results suggest a high therapeutic potential for this novel human monoclonal antibody.

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**Development of potent small-molecule inhibitors of Shiga toxin**

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Tetanus neurotoxin (TeNT) synthesized by the anaerobic *Clostridium tetani* is one of the most deadly bacterial toxins known to humans, and is responsible for the severe neurological illness known as tetanus characterized by generalized muscle spasms and rigid paralysis. TeNT is known to enter motor nerve terminals of the neuromuscular junction and is retrogradely transported along the motoneuron axons to the cell body. TeNT is transcytosed into adjacent inhibitory interneurons where it blocks the release of inhibitory neuro-transmitters (i.e., gamma-aminobutyric acid and glycine), leading to spastic paralysis. Vaccination is the best way to prevent tetanus disease, but its protection does not last forever. Adults should get a tetanus shot, or booster, every 10 years. In the present work we have studied the efficacy of fully human recombinant monoclonal antibodies (Mabs) directed to TeNT, and obtained from B-cells of a recently immunized blood donor, using “single cell PCR” method. This approach entails the isolation of B-cells by a cell sorter coupled to a cytometer using a B-cell selective marker, anti-CD19 associated to a B-cell specific marker, C-ter fragment of TeNT-FITC-labeled. RT-PCR on single cell allowed the conversion of total RNA to cDNA, which was amplified using specific primers to select variable regions of the heavy and light chain of IgG. These variables regions were cloned into an expression vector containing the corresponding constant part of human IgG1. The expression vectors were then transiently transfected into CHO cells before purification of the expressed Mabs. Several new antitetanus Mabs were characterized in order to demonstrate their protective quality as compared to that of the therapeutic human polyclonal antibodies, Gammatumanos (LFB). Mice were injected with different doses of antibodies combined to lethal doses of TeNT. One of the tested Mabs exhibited a remarkable efficacy at nano-gram concentrations. In addition, we have tested the capacity of this human Mab to inhibit the binding and/or translocation of TeNT within motor nerve terminals. For this, we have used a fusion protein composed of the atoxic C-terminal fragment of TeNT coupled to the fluorescent probe Cy3 and isolated mouse neuromuscular preparations with or without the presence of antibodies. Confocal microscopy revealed that the fusion protein rapidly clustered inside motor nerve terminals of the neuromuscular junction under control conditions, and this internalization was markedly reduced when the novel effective human Mab was used. Comparison with Gammatumanos was also performed. Our results suggest a high therapeutic potential for this novel human monoclonal antibody.

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