EPITOPE MAPPING OF A SPHINGOMYELINASE D FROM LOXOSCELES INTERMEDIA SPIDER BY ANTIBODIES ELICITED IN MICE WITH DIFFERENT GENETIC BACKGROUND

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Loxoscelism, caused by *Loxosceles* brown spider, is the most severe form of araneism in Brazil. It is characterized by a necrotic skin ulcer that heals slowly and may require surgical excision or skin grafting and systemic effects as acute renal failure and disseminated intravascular coagulation. Sphingomyelinases D (SMase D) toxins which are responsible for the local and systemic effects induced by *Loxosceles* venoms. These proteins are the most antigenic and immunogenic component of the venom and then important target to produce therapeutics to treat Loxoscelism. Aiming to investigate the immunodominant B-cell epitope of the SMase D toxin, in the present work, human MHC class II transgenic mice (HLA DQ6, DQ8, DR4) were immunized with a recombinant SMase D derrnecrotic toxin (rLiD1his) as well as BALB/c(BC) and C57BL/6(C57) mice. After immunization the sera of the animals were withdrawn and analyzed by ELISA. We observed high antibody titer against rLiD1his and *L. intermedia* venom, but not against BSA, in all the groups except HLA DR4. Using SPOT-synthesis methodology we determined the B-cell epitope recognized by the different animals sera. One epitope region was identified exclusively in transgenic mice and other 2 epitope regions were observed in at least 2 mice strains. To evaluate the capacity of neutralization of derrnecrotic, haemorrhagic and oedematogenic effects of the different mice sera, we pre-incubated sera with rLiD1his and then injected intradermal into naïve rabbits. We observed that, except DR4 mice, all animal groups sera were effective to neutralize rLiD1his toxic effects. In conclusion, LiD1 protein don’t have immunodominant epitope due to the difference observed among the epitopes found to react with the different mice sera. In view of this we can understand better the problem in B-cell epitope prediction that don’t take in account the animal genetic background to make predictions.

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