BTX Applications

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Electroporation used in vaccine against Avian Influenza in mice

A single immunization with HA DNA vaccine by electroporation induces early protection against H5N1 avian influenza virus challenge in mice

Background: Developing vaccines for the prevention of human infection by H5N1 influenza viruses is an urgent task. DNA vaccines are a novel alternative to conventional vaccines and should contribute to the prophylaxis of emerging H5N1 virus. In this study, we assessed whether a single immunization with plasmid DNA expressing H5N1 hemagglutinin (HA) could provide early protection against lethal challenge in a mouse model.
Methods: Mice were immunized once with HA DNA at 3, 5, 7 days before a lethal challenge. The survival rate, virus titer in the lungs and change of body weight were assayed to evaluate the protective abilities of the vaccine. To test the humoral immune response induced by HA DNA, serum samples were collected through the eye canthus of mice on various days after immunization and examined for specific antibodies by ELISA and an HI assay. Splenocytes were isolated after the immunization to determine the antigen-specific T-cell response by the ELISPOT assay.
Results: Challenge experiments revealed that a single immunization of H5N1 virus HA DNA is effective in early protection against lethal homologous virus. Immunological analysis showed that an antigen-specific antibody and T-cell response could be elicited in mice shortly after the immunization. The protective abilities were correlated with the amount of injected DNA and the length of time after vaccination.
Conclusion: A single immunization of 100 μg H5 HA DNA vaccine combined with electroporation was able to provide early protection in mice against homologous virus infection.

Table 1: Protection of mice immunized with HA DNA vaccine against lethal H5N1 virus challenge[a]

		Lung virus titer[b] (log_{10} $TCID_{50}$/ml)			Survival rate (No. of survivors/no. tested)		
Plasmid DNA	Dose (μg)	3 days	5 days	7 days	3 days	5 days	7 days
HA DNA	10	6.25 ± 0.35	4.80 ± 0.28[c]	4.25 ± 0.35[c]	0/12	0/12	6/12
	50	5.75 ± 0.35	4.55 ± 0.07[c]	4.00 ± 0.70[c]	0/12	4/12	10/12[d]
	100	5.75 ± 0.35	4.55 ± 0.07[c]	3.25 ± 0.35[c]	0/12	6/12	12/12[d]
Control			6.25 ± 0.35			0/12	

Table 1: Mice were immunized once with HA DNA 3, 5 and 7 days, respectively, before the challenge and at various dosages. Then all the mice were challenged under anesthesia with 5 LD50 of homologous A/Chicken/Henan/12/2004(H5N1) virus. Three days after the viral challenge, the mice were sacrificed. The trachea and lungs were taken out and washed twice by injecting 2 ml of PBS containing 0.1% BSA. The bronchoalveolar wash was used for virus titration after removing cellular debris by centrifugation. The survival of mice 3 weeks after the challenge was measured. bValues represent means ± SD of 4 mice from each group. c Significantly different from the control groups ($p < 0.05$) by ANOVA. d Significantly different from the control groups ($p < 0.05$) by Fisher's exact test.

ECM® 830 In Vivo Electroporation Protocol

Tissue preparation:
For immunization, the BALB/c mice were anaesthetized with a mixture of ketamine and lobelanine and injected with HA DNA into the right quadriceps muscle. 5mm 2-Needle array electrode was then inserted into the muscle to cover the NDA injection sites

Electroporation Settings:

Voltage:	100 V
Pulse length:	50 ms
Number of pulses:	3
Electrode	5mm 2-Needle Array

Switch positive and negative leads to change polarity and repeat electroporation settings.

Reference;
Zheng, L., Wang, F., Yang, Z,, Chen, J,. Chang, H., Chen, Z, A single immunization with HA DNA vaccine by electroporation induces early protection against H5N1 avian influenza virus challenge in mice. *BMC Infectious Diseases* (2009) **9**(17)

Part Numbers

ECM 830 System – 450002

Two Needle Array 5mm - 450168

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