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Immunological evaluation of a dna cocktail vaccine with co-delivery of Calcium phosphate nanoparticles (capns) against the *Toxoplasma gondii* RH strain in BALB/c mice

Ahmad Daryani¹, Mohammad Taghi Rahimi², Shahabeddin Sarvi¹, Mahdi Sharif¹, Ehsan Ahmadpour³ and Alireza Khalilian⁴

¹Mazandaran University of Medical Sciences, Iran

²Shahroud University of Medical Sciences, Iran

³Tabriz University of Medical Sciences, Iran

⁴Mazandaran University of Medical Sciences, Iran

Many recent studies have been conducted to evaluate protective immunity mediated by DNA vaccines against toxoplasmosis. Cocktail DNA vaccines showed better immune responses compared to single vaccines. The objective of the current study was to evaluate the protective efficacy of rhomboid 4 (ROM4) and cocktail DNA vaccines (ROM4 + GRA14) of the *Toxoplasma gondii* RH strain with or without coated calcium phosphate nanoparticles (CaPNs) as the adjuvant to improve the immunogenicity against the *T. gondii* RH strain in BALB/c mice. Cocktail DNA vaccines of pcROM4+ pcGRA14 of the *T. gondii* RH strain were constructed. CaPNs were synthesized and the cocktail DNA vaccine was coated with the adjuvant of CaPNs. Immunogenicity and the protective effects of cocktail DNA vaccines with or without CaPNs against lethal challenge were evaluated in BALB/c mice. pcROM4 and cocktail DNA vaccine coated with CaPNs significantly enhanced cellular and humoral immune responses against *Toxoplasma* compared to pcROM4 and cocktail DNA vaccine without CaPNs ($p < 0.05$). These findings indicate that the survival time of immunized mice after challenge with the RH strain of *T. gondii* was increased compared to that of controls and the DNA vaccine provided significant protection in mice ($p < 0.05$). The CaPN-based cocktail DNA vaccine of pcROM4 + pcGRA14 showed the longest survival time compared to the other groups. Co-immunization with CaPN-based cocktail DNA vaccine (pcROM4 + pcGRA14) boosted immune responses and increased the protective efficacy against acute toxoplasmosis in BALB/c mice compared to both single gene and bivalent DNA vaccine without nano-adjuvants.

daryanii@yahoo.com