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PILAIWAN HUTAMEKALIN : DEVELOPMENT OF ANTI-G-PROTEIN ANTIBODIES BY GENETIC IMMUNIZATION. THESIS ADVISOR: STEFANO O. CASALOTTI Ph.D., PIYARAT GOVITRAPONG Ph.D., SAKOL PANYIM Ph.D., NAIPHINICH KOTCHABHAKDI Ph.D., ALBERT KETTERMAN Ph.D.
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DNA immunization is a novel method for inducing immune response against antigens. This technique is based on the concept of injecting animals with a plasmid capable of expressing in situ the antigenic protein of interest. The advantages of this technique are the induction of both humoral and cellular responses, decrease time and cost of production. The aim of this work was to determine whether this procedure would also be useful for the production of antibodies for biomedical and neuroscience research.

The GTP-binding regulatory proteins (G proteins) were selected as target model for the production of antisera because they represent a typical family of proteins involved in a variety of neuronal functions and for which a battery of subunit specific antibodies would be very useful. The cDNA sequences for, $G_{\alpha o}$, $G_{\alpha i1}$, and $G_{\beta 2}$ were amplified by reverse transcriptase polymerase chain reaction (RT-PCR) and ligated into pCI-neo plasmid. Following DNA sequencing confirmation of the authenticity of the inserts. 50 μg of plasmid DNA were injected into each tibialis anterior (TA) muscle of mice and rabbits. Sera were collected at various time during the trials, before and after injection with the plasmid constructs. The immune responses were assessed by 12% SDS-PAGE gel of rat tissue samples and partially purified recombinant $G_{\alpha o}$ subunit. The sera of the mice injected with any of the plasmids did not reveal any specific band as compared to pre-immune serum. The $G_{\alpha o}$, and $G_{\beta 2}$, rabbit antisera recognized bands with molecular weight of 34±5 kDa and 45±9 kDa, respectively. Further characterization of the $G_{\alpha o}$ serum indicated that the relative intensity of the specifically stained band in different rat tissues was similar to the know relative distribution of $G_{\alpha o}$ protein in those tissues. This work indicated that genetic immunization is a suitable alternative methodology for the production of antibodies for research, however further optimization of the technique is required.