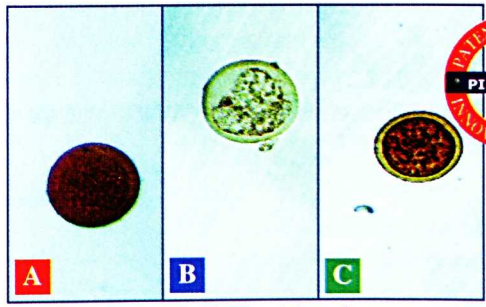
IMURON™

A Total Solution for Maximising Antibody Response

DNA vaccination leads to intracellular production of proteins, which are endogenous antigen in nature. It favours the specific type of immune response, which is mediated by the Major Histocompatibility Complex (MHC) class I pathway, resulting a strong antigen-specific cytotoxic T lymphocytes (CTL) response. In contrast, the protein vaccination is bias towards the MHC class II pathway and the antigens encountered are exogenous.

CD8+ cytotoxic T cells are more effective in eliminating viruses that are protected by the cell they reside in. T cells do not neutralize virus particles or viral antigens that are present outside of the cells. Antibodies produced by the host bind and form a complex with extracellular viruses or antigens. If virus particles are the target antigens, the antigen-antibody binding will lead to the neutralization of virus from infecting host cells. Therefore, antibodies are important in eliminating infectious viruses or antigens from infecting host cells. However, the level of antibodies generated following vaccination with DNA vaccines is relatively low.

Several Approaches have been taken to further improve the humoral response of DNA vaccines without suppressing the T cell (CTL) response. For instance, improvement of plasmid backbone and injection vehicles to increase expression of antigen in muscle, co-injection with plasmid encoding co-stimulating molecules, cytokines or chemokines (Pasquini *et al.* 1997; Kim and Weiner, 1997), and the use of plasmid DNA encoding fusion proteins to facilitate antigen targeting or influence antigen localization (Planelles *et al.* 2001).



Positive immunoperoxidase staining of rat oocytes by antisera raised to (A) IMURONTM-ZP3 (B) empty IMURONTM, as negative control (C) rec-ZP3 as positive control, collected 1 month after the first injection. Antisera used were at a dilution of 1:50.

In the research conducted, the concept of fusion gene is employed to improve antigen processing and presentation. Nucleoprotein (NP) gene (from viruses of family *Orthomyxoviridae* or *Paramyxoviridae*) is chosen because NP DNA vaccines has successfully induced strong CTL responses and at the same time producing a high level of antigen specific antibodies, mainly IgG2a subclass via cross priming (Yankauckas *et al.* 1993). It is used as an immunoenhancer and can assure that an increase in the level of antibody production does not suppress the development of CTL responses. Unlike cytokine genes, enhancement of antibody immune response is sometimes accompanied by a suppression of cell-mediated immune response and also inhibition in the expression of immune memory response following administration of IL-4.

ZP3 (zona pellucida 3) DNA vaccine is a good example. Antibody against ZP3 protein was too low to be detected by ELISA despite of three intramuscular injections (booster doses) with ZP3 DNA vaccine (50-100 µg/animal). As most of the DNA vaccines, ZP3 vaccine has preference towards cell-mediated immune response. When ZP3 protein is genetically conjugated with NP protein, production of antibodies against ZP3 protein has increased substantially up to more than 100 folds.

The NP gene was cloned into pcDNA3 using *Hind*III and *Eco*RI sites to yield IMURON™. The IMURON™ was designed to express NP conjugated protein. The termination codon of NP gene was replaced with HIS_6 coding sequence and limited number of enzyme restriction sites via PCR. HIS-tag functions as reporter gene while the enzyme restriction sites facilitate cloning of second gene, the gene of interest.

To test the efficacy of IMURON™, the 5′ terminus of ZP3 gene was ligated to the 3′ terminus of the NP DNA sequence at *Eco*RI restriction site. The resultant recombinant was denoted as IMURON™-ZP3.

IMURON™ is a patent pending PI20023353 invention.

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