INVITED REVIEW

Antiparasitic DNA vaccines in 21st century

Halina Wedrychowicz
Department of Molecular Biology, Laboratory of Molecular Parasitology, W. Stefani Institute Parasitology, Polish Academy of Sciences, 51/55 Twarda St., 00-818 Warsaw, Poland

Abstract

Demands for effective vaccines to control parasitic diseases of humans and livestock have been recently exacerbated by the development of resistance of most pathogenic parasites to anti-parasitic drugs. Novel genomic and proteomic technologies have provided opportunities for the discovery and improvement of DNA vaccines which are relatively easy as well as cheap to fabricate and stable at room temperatures. However, their main limitation is rather poor immunogenicity, which makes it necessary to couple the antigens with adjuvant molecules. This paper review recent advances in the development of DNA vaccines to some pathogenic protozoa and helminths. Numerous studies were conducted over the past 14 years of 21st century, employing various administration techniques, adjuvants and new immunogenic antigens to increase efficacy of DNA vaccines. Unfortunately, the results have not been rewarding. Further research is necessary using more extensive combinations of antigens; alternate delivery systems and more efficient adjuvants based on knowledge of the immunomodulatory capacities of parasitic protozoa and helminths.

Keywords
DNA vaccines, adjuvants, exprimental trials, immunomodulation

Introduction

In our previous review concerning usefulness of cDNA vaccination in preventing parasitic diseases of man and animals (Kofta and Wedrychowicz 2001), we concluded that this technology opens up many new opportunities but still requires much more research like cloning and testing antigenic properties and protectivity of new c-DNA sequences, using different ways of delivery, design of vectors containing appropriate immunomodulatory sequences, the coadministration of immunomodulating DNA constructs in order to trigger protective immune mechanisms not necessarily the same as those elicited during natural infection. During the past thirteen years most of these suggestions have been addressed in many vaccination trials but still there is no anti-parasitic DNA vaccine available on the market.

Vaccination is the most effective and efficient procedure for disease prevention (McCullers 2007). At the end of XX century development of molecular biology and biotechnology raised hopes for a quick development of anti-parasitic vaccine industry thanks to DNA (cDNA) based vaccines (Wolff et al. 1990). However, despite that numerous experimental studies have been conducted since Wolff’s publication, to date there are only few DNA vaccines that have been approved for veterinary use (Davidson et al. 2005; Garver et al. 2005; Bergman et al. 2006; Person et al. 2008). Moreover, only two of them are prophylactic vaccines; one to prevent West Nile Virus infections in horses (Davidson et al. 2005) and the second to stimulate innate and adaptive immune responses of salmons to infections with haematopoietic necrosis virus (Garver et al. 2005). Despite the success of these DNA vaccines and the positive results of others in clinical trials, the efficiency of DNA vaccines in humans and large animals like bovines and sheep is still lower than it was expected (Liu 2010).

DNA vaccination: mechanisms of action and adjuvants

Several factors still limit the effectiveness of vaccination, which must be overcome with the advances in the biotechnology field and a deeper comprehension of the immune mechanisms active during parasitic infections. It is commonly agreed that an ideal vaccine should be save for entire popula-
ation as well as induce a long term immunity and demonstrate a good, long lasting efficacy. It also should be efficient after single dose application, be easy for administration, simple to produce, resistant to temperature changes, multivalent and able of controlling disease (Levine and Sztein 2004).

DNA vaccines are composed of an antigen-encoding gene or cDNA and a strong mammalian promoter expressed on a plasmid backbone of bacterial DNA (Wolf et al. 1990; Sato et al. 1996; Klinman et al. 1997). These plasmids contain DNA sequences necessary for selection and replication in bacteria and often additional promoters, enhancers, and other elements designed to increase expression of the encoded protein in vaccinated organism. When administrated, DNA vaccine plasmid is absorbed by the cells then uses a net of microtubules and associated with them motor proteins in the cytoplasm to reach the cellular nucleus (Vaughan and Dean 2006). Cells transfected with DNA vaccines transcribe, translate, and express the encoded protein(s) in the context of MHC of vaccinated organism. Transcription and translation of the transgene occurs via the host's cellular machinery and the produced proteins are then presented to the surface of cells to become a target of the immune system. Dendritic cells are probably the most important antigen presenting cells associated with the capture and processing of antigens via receptor-mediated endocytosis and its presentation to MHC class I and II. CD4+ and CD8+ lymphocytes can be activated during the process of DNA vaccination, inducing cellular immune and specific antibodies responses (Payette et al. 2001; You et al. 2001).

Multiple phase I clinical trials involving DNA vaccines against viral and bacterial infections have been conducted (Martin et al. 2008). Results from those trials indicate that although DNA vaccines seem to be safe, the immune response they elicit in humans and large mammals is poor (Mancini et al. 2005; Martin et al. 2008).

Efforts have been made to improve immunogenicity of DNA vaccines by changing promoters, codon usage of antigen sequences (Zhu et al. 2010), the insertion of genetic adjuvants such as cytokines and innate immune activation molecules, strategies to prime and boost vaccination, and the route of administration (Saade and Petrovsky 2012).

Candidate genes for a DNA prophylactic anti-parasitic vaccine construction, which are usually molecules associated with pathogenicity and/or important for parasite feeding, reproduction and survival in the host can be modified to target proteins to different cellular locations: cytoplasm, cell wall or extracellular medium, since the expression of proteins in different compartments can influence the immunological response. Moreover, targeting antigens of interest to proteasomes or endosomes, using ubiquitin fusions, can also increase the number of peptides available to ligate to the major histocompatibility complex of class I (MCH-I) when induction of cytotoxic cells is required (Dobano et al. 2008).

Often, when it is necessary to express more than one gene of interest to trigger a protective immune response, polycistronic expression systems or even molten epitopes expressed as a unique polypeptide can be used (Rainczuk et al. 2004; Yuan et al. 2006; Anand et al. 2011; Zhu et al. 2011). Immune stimulatory sequences, like unmethylated phosphodiester linked cytosine and guanine (CpG) motifs which interact with the Toll-like receptor 9 (TLR-9) may induce a series of immune stimulatory cytokines that lead to the activation of B-cells, monocytes, macrophages, dendritic cells (DCs) and natural killer (NK) cells, enhancing both non-specific and antigen-specific responses (Kennedy et al. 2006; Jenkins et al. 2004). In turn, vaccination with constructs encoding CTLA-4 fusion proteins (which bind to CD80/86 of APC’s) can induce strong antibody responses and provides a novel generic DNA vaccine for the development of therapies against a wide range of diseases. Kennedy et al. (2006) investigated the ability of ovine cytotoxic lymphocyte antigen 4 (CTLA-4) mediated targeting and ruminant specific CpG optimised plasmids, both alone and in combination, to enhance immune responses in sheep to the pro cathepsinB(FhCatB) antigen from Fasciola hepatica. They found that CTLA-4 mediated targeting enhanced the speed and magnitude of the primary antibody response and effectively primed for a potent memory response compared to conventional DNA vaccination alone, which failed to induce a detectable immune response. While the CpG-augmentation of the CTLA-4 targeted construct did not further enhance the magnitude or isotype profile of the CTLA-4 induced antibody titres, it did result in the induction of significant antigen-specific, lymphocyte-proliferative responses that were not observed in any other treatment group, showing for the first time that significant cellular responses can be induced in sheep following DNA vaccination. In contrast, CpG-augmentation in the absence of CTLA-4 mediated targeting failed to induce a detectable immune response. However, Januszkiewicz (2010) did not observed any significant differences in cellular and humoral responses after invasion of Fasciola hepatica in Merino lambs vaccinated intramuscularly with cDNA encoding F. hepatica phosphoglycerate kinase (FhPGK) together with ovine CTLA-4 in comparison to non vaccinated animals. Although fluke burdens were similar in vaccinated and control sheep, some statistically significant differences were observed in fluke body size. The highest number of flukes in size between 0.5 to 1.5 cm was observed in group vaccinated with cDNA of FhPGK but the number of biggest flukes in this group was the least. Moreover the percentage of initial body weight increase was highest in this group in comparison to control.

A great advantage of DNA vaccines is their ability to polarise immune response of vaccinated organism into TH1 or TH2 regulated profiles not only by modifications to the form of antigen expressed (i.e. intracellular vs. secreted), the method and route of delivery, and the dose of DNA delivered, but also by the co-administration of "genetic adjuvants". Such adjuvants are composed of plasmid DNA encoding immune regulatory molecules such as cytokines, lymphokines or other co-stimulatory molecules and can be administered as a mixture of 2 separate plasmids, one encoding the immunogen and

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the other encoding the cytokine; as a single bi- or polycistron vector, separated by spacer regions; or as a plasmid-encoded fusion protein. The genetic adjuvants have been very often used in experimental trials of DNA vaccines against protozoan infections (Tables: I–III), and rather seldom in research on DNA vaccination against fluke or nematode infections (Table: V, VI).

**DNA vaccination: methods of delivery**

There are various means of delivery of DNA vaccines against parasites (Tables I–VI). It has been known from the start of research on naked DNA vaccines that the outcome of vaccination often depends on the route of the immunisation (Kofta and Wedrychowicz 2001).

The intradermal injection of the c-DNA of the given antigen induces the Th1-dependent response, while injecting of the same antigen in the protein form generates the Th2 response. Naked DNA plasmid transfection is a simple and direct method, free of complex formulations or from agents, to transfer in vivo DNA gene sequences of interest. Yu et al. (2010) investigated BALBc mice response to intranasal or intramuscular vaccination with recombinant pVAX1 plasmids. DNA sequences of Cp12 and Cp21 surface proteins on the sporozoite of Cryptosporidium parvum have been used as antigens. DNA sequences of Cp12, Cp21, Cp12-Cp21, and C (CpG oligodeoxynucleotide (ODN))-Cp12-Cp21 were amplified and then cloned into pVAX1 vector to form the four recombinant plasmids pVAX1-Cp12, pVAX1-Cp21, pVAX1-Cp12-Cp21, and pVAX1-C-Cp12-Cp21. All the four DNA vaccines elicited significant antibody responses and specific cellular responses when compared to control mice that received vector only or PBS. Among those four plasmids, pVAX1-C-Cp12-Cp21 elicited significantly higher levels of IgG. Also, the percentages of CD4+ and CD8+ T cells were significantly higher in the group with pVAX1-C-Cp12-Cp21 nasal sprays. Their efficacy in immunoprotection against homologous challenge was also detected after administration of the four DNA vaccines. The results showed that mice in the pVAX1-Cp12-Cp21 nasal group had a 77.5% reduction in the level of oocyst shedding and a significant difference was detected when this group was compared with the pVAX1, PBS, pVAX1-Cp12, and pVAX1-Cp12 groups. The reduction in the level of oocysts shedding from the group of pVAX1-Cp12-Cp21 nasal spray was also higher than that of pVAX1-Cp12-Cp21 group. However, to achieve significant levels of immunity in humans and large animals, DNA delivery methods often require very high doses of plasmids and multiple doses (Wahren and Liu 2014) thus, increasing the efficiency of DNA vaccines in humans is still required. Insufficient cell membrane permeability and low cellular uptake of DNA plasmid vectors contribute for a decreased protein expression and consequently for a reduction of DNA vaccine effectiveness.

Liposomes are synthetic vesicles consisting of phospholipid bilayers and represent one of the major techniques used for gene delivery into cells nowadays. A large number of cationic lipids with different molar ratios, such as derivate of diacylglycerol, lipids, polyamines and cholesterol, make the generation of different kinds of liposomes, possessing different physicochemical characteristics like size and net surface charge, possible (Hiszczynska-Sawicka et al. 2011 a,b). Upon mixing with cationic liposomes, plasmid DNA is condensed into lipopplexes that trigger cellular uptake and facilitate the release of DNA from intracellular vesicles.

Bacterial DNA vaccine delivery systems consist in the internalization of bacteria, harboring a plasmid vector containing the sequence of the gene of interest, by target cells. Subsequent primary vesicles are formed and then fused to lysosomal compartments where lysis of bacteria occurs, releasing the plasmid DNA into the host’s cytosol. The plasmid DNA then migrates to the nucleus of the cell where the gene sequence of interest is transcribed for subsequent transduction and protein synthesis by the host’s cells machinery (Du and Wang 2005). The use of bacteria as vehicles for the delivery of DNA vaccines has several advantages when compared to other methods. Bacteria deliver DNA vaccine plasmids directly into the interior of the cells protecting the DNA from degradation by nucleases.

The DNA prime/vector boost concept, has been initially used mostly in research on vaccine against malaria (Schneider et al. 2001; Kimani et al. 2014). DNA priming appears to improve the outcome of boosting with recombinant proteins, or with vector-based vaccines. The potency is dependent upon DNA being the prime rather than the boost. Although the mechanism is still not entirely clear, it is possible that focusing the immune response on the one or few antigens generated by the plasmid gene(s), may result in potent boosting when larger amounts of proteins are produced by the viral vector in the context of the innate/inflammatory responses generated by the viral vector (Wahren and Liu 2014). Kimani et al. (2014) used heterologous prime-boost immunization strategy, employing a chimpanzee adenovirus vector followed by modified vaccinia Ankara (MVA), both encoding the pre-erythrocytic malaria antigen ME-thrombospondin-related adhesive protein (TRAP), to vaccinate adults in Kenya and The Gambia in areas of similar seasonal malaria transmission. The vaccination induced strong cellular and humoral immune responses. This prime-boost approach targeting the pre-erythrocytic stage of the malaria life-cycle is now being assessed for efficacy in a target population.

The prime /boost strategy has been widely used in experimental studies on development of effective vaccines against protozoan infections like Leishmania donovani, L. Infantum, Plasmodium falciparum (Table I), Neospora caninum, Toxoplasma gondii (Table II) as well as flukes such as Schistosoma mansoni (Table IV) and a nematode Brugia malayi (Table VI).
| Parasite         | Host     | cDNA                        | Vaccination     | Results                      | Reference          |
|------------------|----------|----------------------------|-----------------|------------------------------|--------------------|
| B. gibsoni       | dog      | P29                        | prime/boost     | reduced parasitemia          | Fukumoto et al. 2009 |
| B. gibsoni       | dog      | BgGARP                     | prime/boost     | reduced parasitemia by 78%   | Cao et al. 2013    |
| L. donovani      | hamster  | ribosomal P1 gene          | prime/boost     | reduced parasitemia          | Masih et al. 2011  |
| L. infantum      | mouse    | C-terminal of cysteine peptidase 1 | prime/boost | no protection              | Rafati et al. 2008 |
| L. major         | mouse    | SLA + IL-12                | i.d.            | moderate protection         | Yamakami 2001     |
| L. major         | mouse    | VP22-amastin-EGFP          | i.d.            | partial protection          | Bolhassani et al. 2011 |
| L. major         | mouse    | LACK + IL-22               | i.m.            | 80% protection              | Hezarjaribi et al. 2013 |
| T. b. brucei     | mouse    | invariant surface glycoprotein | i.m.            | partial protection          | Cruz Lança et al. 2011 |
| T. evansi        | mouse    | beta tubulin               | i.m.            | partial protection          | Kurup and Tewari 2012 |
| P. chabaudi      | mouse    | AMA-1 + MSP4/5             | gene-gun        | variable protection         | Rainczuk et al. 2004 |
| P. falciparum    | man      | ME-TRAP                    | prime/boost     | partial protection          | Dunachie et al. 2006 |
| P. falciparum    | man      | CS                         | prime/boost     | no protection               | Dunachie et al. 2006 |
| P. yoelli        | mouse    | PyCSP or PyHEP17 + Ub or LAMP** | i.m.            | no increase in protection   | Dobano et al. 2007 |

i.d. = intradermal, i.m. = intramuscular, prime/boost = first vaccination with DNA of the antigen and second with protein
**Ub = Ubiquitin gene, LAMP = lysosome-associated membrane protein gene

| Parasite   | Host   | cDNA                        | Vaccination | Results                                      | Reference          |
|------------|--------|-----------------------------|-------------|----------------------------------------------|--------------------|
| C. parvum  | mouse  | C-Cp12-Cp21-DNA             | i.n.        | lower oocyst shedding                        | Yu et al. 2010     |
| N. caninum | mouse  | NeSAG1- and NeSRS2          | prime/boost | effective protection                         | Cannas et al. 2003 |
| N. caninum | mouse  | NeGRA7 or NcsHSP33         | i.m.        | partial protection against congenital neosporosis | Liddell et al. 2003 |
| N. caninum | mouse  | BAG1 and MAG1               | i.m.        | effective protection                         | Nielsen et al. 2006 |
| T. gondii  | mouse  | SAG1-ROP2 + pIL-12          | i.m.        | IL12 enhanced protection                    | Xue et al. 2008a   |
| T. gondii  | mouse  | SAG1, ROP2 and GRA2         | i.m.        | potent, long lasting protection              | Xue et al. 2008b   |
| T. gondii  | mouse  | TgSAG1 + IL18               | i.m.        | protection against infection                | Liu et al. 2010    |
| T. gondii  | sheep  | GRA1,4,6,7 + liposomes      | i.m.        | significant antibody response                | Hiszczynska-Sawicka |
| T. gondii  | sheep  | pROP1-CD154                 | i.m.        | Th1/Th2 response, ROP1 alone – Th1 response | et al. 2011a, b    |
| T. gondii  | mouse  | SAG1 and MIC4               | i.n.        | increased survival                          | Wang et al. 2009   |
| T. gondii  | mouse  | MIC3                        | i.d.        | effective protection against T. gondii challenge | Xiang et al. 2009  |
| T. gondii  | mouse  | perforin1+IL18              | i.m.        | increased survival time, strong immune response | Yan et al. 2011    |
| T. gondii  | mouse  | AMA1                        | prime/boost | increased survival rate, better immune response prolonged survival time, Th1 | Yu et al. 2012     |
| T. gondii  | mouse  | MIC3 and ROP18              | i.m.        | cellular response                            | Qu et al. 2013     |

i.n. = intranasal, i.m. = intramuscular, i.d.= intradermal, prime/boost = first vaccination with DNA of the antigen and second with protein
### Table III. Selected DNA vaccination trials against chicken coccidiosis in years 2001-2013

| Parasite      | Age of chicken | cDNA          | Vaccination   | Results                                                                 | Reference               |
|---------------|----------------|---------------|---------------|------------------------------------------------------------------------|-------------------------|
| *E. tenella*  | 3 days         | S401in S. typhimurium | oral         | 55–57.5% protection against *E. tenella* challenge                      | Du and Wang 2005        |
| *E. tenella*  | 14 days        | TA4 + chicken IL-2     | i.m.          | decreased oocyst shedding, better weight gains                         | Xu *et al.* 2008        |
| *E. tenella*  | 14 days        | TA4 + chicken IL-2     | i.m.          | cross-protection to *E. necatrix* and *E. acervulina*                  | Song *et al.* 2009      |
| *E. acervulina* | 14 days       | cSZ-2                | i.m.          | cross-protection against *E. tenella*                                  | Shah *et al.* 2010      |
| *E. tenella*  | 14 days        | MZ5-7 + chicken IL-17 | i.m.          | partial protection to *E. tenella*                                     | Gherletu *et al.* 2011  |
| *E. acervulina* | 14 days       | cSZ2+chIL-2 +chIFNc  | i.m.          | cSZ-2+chIL-2 DNA partial protection                                    | Shah *et al.* 2011      |
| *E. tenella*  | 14 days        | SO7 + chIL-2         | i.m.          | decreased caecal lesions, partial protection                            | Song *et al.* 2013      |
| *E. acervulina* | 14 days       | 3-1E gene            | i.m.          | pcDNA3-1E was safe to chicken and environment                         | Zhao *et al.* 2013      |
| *E. tenella*  | 14 days        | EtMIC2 + chIL18      | i.m.          | increased weight gain, decreased oocyst shedding                       | Shi *et al.* 2014       |

i.m. = intramuscular, chIL = chicken interleukine gene, chIFN = chicken interferon

### Table IV. Selected DNA vaccination trials against *Schistosoma* sp. infections in years 2001-2014

| Parasite       | Host          | cDNA              | Vaccination | Results                                                                 | Reference               |
|----------------|---------------|-------------------|-------------|------------------------------------------------------------------------|-------------------------|
| *S. japonicum* | mouse         | Sj23              | i.m.        | specific IgG antibodies, no protection against challenge              | Waine *et al.* 2002    |
| *S. japonicum* | bufallo       | SjCTPI + Hsp70    | i.m.        | worm burden reduced by 51.2%, egg hatching by 52%                     | Da’Dara *et al.* 2008   |
| *S. japonicum* | bufallo       | Sj23 + Hsp70      | i.m.        | reduced worm burden by 50.9%, egg hatching by 52%                    | Da’Dara *et al.* 2008   |
| *S. japonicum* | mouse         | Sj14+Sj23         | i.m., i.d., s.c | protective immunity above 50%, granuloma reduction                     | Yuan *et al.* 2006      |
| *S. japonicum* | mouse         | SjGST + SjMLP/hsp70 | i.m. | SJGST combined with SjMLP/hsp70- reduction of worm burden by 31.31% and eggs number by 58.59% | He *et al.* 2010        |
| *S. japonicum* | mouse         | Sj26GST + CIM     | i.m.        | reduced egg number by 79%, fluke burden by 68.4%                     | Li *et al.* 2011        |
| *S. mansoni*   | mouse         | Sm32              | i.v.        | reduction in fecundity by 37%                                        | Chlichlia *et al.* 2001 |
| *S. mansoni*   | mouse         | Sm23              | i.d.        | levels of protection 31–34%                                          | Da’dara *et al.* 2002   |
| *S. mansoni*   | mouse         | Sm-p80            | i.m.        | 59% reduction in worm burden, 84% in egg production                   | Ahmad *et al.* 2009a    |
| *S. mansoni*   | baboon        | Sm-p80            | i.m.        | reduction in egg production by 32%                                    | Ahmad *et al.* 2009b    |
| *S. mansoni*   | mouse         | Sm-p80            | prime/boost  | egg production reduced up to 75%, worm burden by 70%                  | Ahmad *et al.* 2009c    |

i.m. = intramuscular, i.d. = intradermal, s.c. = subcutaneous, i.v. = intravenous, prime/boost = first vaccination with DNA of the antigen and second with protein
Table V. Selected cDNA vaccination trials against liver fluke infections in years 2001-2014

| Parasite   | Host | cDNA   | Vaccination | Results                                                                 | Reference                  |
|------------|------|--------|-------------|------------------------------------------------------------------------|----------------------------|
| C. sinensis| rat  | CsFABP | i.d.        | decreased worm burden by 40.9%, IgG2a, IFN-gamma                        | Lee et al. 2006a           |
| C. sinensis| rat  | CsCP   | i.d.        | decreased worm burden by 31.5%, IgG2a, IFN-gamma                        | Lee et al. 2006b           |
| F. hepatica| mouse| FABP   | i.d. i.m.   | injection induced mixed Th1/Th2 response                                | Smooker et al. 2001        |
| F. hepatica| mouse| CatL   | i.m.        | predominantly Th2 related response (IgG1)                               | Smooker et al. 2001        |
| F. hepatica| rat  | GST    | i.m.        | decreased worm burden by 61–75%, Th2 response                          | Wedrychowicz et al. 2002  |
| F. hepatica| rat  | GST    | i.m.        | decreased worm burden by 54%, IgG2b                                    | Wedrychowicz et al. 2003  |
| F. hepatica| sheep| FhCatB+CTLA4 | i.m.      | enhanced immune response following vaccination                          | Kennedy et al. 2006        |
| F. hepatica| sheep| FhPGK+CTLA4 | i.m.      | better weight gain after challenge infection                            | Januszewicz 2010           |
| F. hepatica| rat  | PGK    | i.m.        | fluke burden reduced by 48–55%                                         | Jaros et al. 2010          |
| F. hepatica| mouse| FhSAP-2 | i.m.        | fluke burden reduced by 83.3%                                          | Espino et al. 2010         |
| F. hepatica| mouse| CTLA4Cat B2 | i.m.    | enhanced antibody response than without CTLA4                           | Jayaraj et al. 2012        |
| F. hepatica| mouse| p MCP3 Cat B2, | i.m.     | higher antibody avidity than without MCP3                              | Jayaraj et al. 2012        |
| F. hepatica| rat  | FhPcW1 | i.m.        | decreased fluke burden by 19.4%                                        | Wesolowska et al. 2013     |

Table VI. Selected cDNA vaccination trials against parasitic nematodes in years 2001-2014

| Parasite  | Host | cDNA   | Delivery | Results                                                                 | Reference                  |
|-----------|------|--------|----------|------------------------------------------------------------------------|----------------------------|
| B. malayi | jird | Bm-ALT2| DNA      | 57 % of protection, Th1 regulated response                              | Thirugnanam et al. 2007   |
| B. malayi | jird | Bm-ALT2| prime/boost | 64% of protection, Th1/Th2 response                                    | Thirugnanam et al. 2007   |
| B. malayi | mouse| BmALT2+BmVAL1 | prime/boost | 82% protection, IgG1, IgG2a, IgG3 responses                             | Kalyanasundaram et al. 2011|
| B. malayi | jird | BmALT2+BmVAL1 | prime/boost | reduction of worm burden by 85%                                        | Kalyanasundaram et al. 2011|
| B. malayi | jird | BmALT2+BmVAH | DNA       | 57% of protection, IgG2a, IgG2b                                        | Anand et al. 2011          |
| B. malayi | mouse| BmTPX  | DNA      | 37% of protection, IgG2a, IgG2b, IgA                                  | Anand et al. 2008          |
| H. contortus | goat | HcGPX  | DNA      | epg reduced by 36%, worm burden by 35.6%                               | Sun et al. 2011            |
| H. contortus | goat | H11 and IL-2 | DNA     | epg reduced by 56.6%, worm burden by 46.7%                             | Zhao et al. 2012           |
| H. contortus | goat | HcGAPDH | DNA      | epg reduced by 34.9%, worm burden by 37.7                              | Han et al. 2012            |
| H. contortus | goat | Dim-1  | DNA      | epg reduced by 45.7%, worm burden by 51.1%                             | Yan et al. 2013            |
| A. ceylanicum | hamster | Ace-MEP-6 | DNA     | worm burden reduction by 80%                                           | Wisniewski et al. 2013    |

Epg = egg per gram of faeces; prime/boost = first vaccination with DNA of the antigen and second with protein
DNA vaccination against parasites – impediments

Specific studies relating to the use of DNA vaccines to immunise against parasitic diseases have mostly concentrated on vaccines against intracellular parasites, i.e. protozoans. Some of the interesting results obtained are presented in Tables I–III.

Starting from year 2001 there were more than 35 reports on c-DNA vaccination against helminth infections and most have been conducted in laboratory animal models (Tables IV–VI). Results depended on the host–parasite system tested and the c-DNA fragments used. Schistosoma trials brought results ranging from 0 to 63% in terms of protection level. In our own research we tested a DNA vaccine against the liver fluke Fasciola hepatica infection in rats, a recognised model for the infection of cattle, and sheep with variable success (Wedrychowicz et al. 2002, 2003; Jaros et al. 2010; Wesolowska et al. 2013).

Why is it so hard to develop a modern vaccine against parasitic protozoa or helminths? Possible reasons lay both on the parasite and host sides. Parasites have developed a number of immune evasion mechanisms, and it is possible that one or more of these played a role in limiting the efficacy of the vaccine under study, including the ability to cleave immunoglobulin, thus neutralising protective antibodies. F. hepatica is known to secrete a number of different cysteine proteases during their development, some of which may act as smoke screen antigens distracting/interfering with host immune responses to critical epitopes on other proteases, while redundancy through overlapping specificities between proteases may also confer some degree of protection (Dalton et al. 2013; Robinson et al. 2013; VanRiet et al. 2007). Several studies have shown that helminths can influence vaccine efficacy by modulating host immune response, in particular when Th1-like and cellular-dependent responses are required (Mc Neilly and Nisbet 2014). Recent studies are showing that infection with helminth parasites alters the bacterial composition of intestinal flora and that the presence or absence of a single microbial species in the gut can regulate the balance between effector and regulatory T cells (Molloy et al. 2012). Although F. hepatica only spends a relative short time traversing the gut wall the parasite may impact on the gut bacterial flora; even after 14 weeks of infection when parasites are residing in the bile ducts enhanced responses to antigen stimulation and increased numbers of immunocytes (e.g. eosinophils) can be observed in the lamina propria.

Mucosal immune system plays an essential role in maintaining intestinal homeostasis with commensal bacteria and other organisms. Gastrointestinal parasites have coevolved with the mammalian immune system similarly to the gut microbiota. Just as commensal bacteria can shape mammalian immunity, helminths exert immune regulatory effects on their mammalian hosts. However, the relationship between helminths and gut microbiota is still unclear. Recent evidence has suggested a role for the cytokine IL-22, during helminth infection and in maintaining mucosal barrier function. IL-22 may therefore play an important role in the relationship between the mammalian immune response, gut microbiota and helminth infections (Molloy et al. 2012).

It has been recently demonstrated that host gender contributes to the ultimate outcome of vaccination against parasites (Wedrychowicz et al. 2003; Wesolowska et al. 2013). It becomes apparent that the differences between the sexes must be taken into account when developing not only new immunoprophylactic strategies but also drugs directed against F. hepatica. Currently the majority of F. hepatica research is carried out using male rats or sheep as they lack periodic fluctuations of hormonal cycle. Nevertheless, the effectiveness of an animal treatment can be influenced by the hosts gender and may not be successful in both sexes. Further, farmed females are often of greater economic interest in animal husbandry than males, e.g. dairy cattle, and research should also focus on them. Taken together, recent data highlights the necessity of research on both sexes in experiments when developing control methods against parasitic infection.

Conclusion

DNA vaccines showed several advantages like antigen presentation by both MHC class I and class II molecules; ability to polarise to TH1 regulated and antigen specific immune response; simplicity of production; stability for storage and shipping; cost-effectiveness. In vivo expression in vertebrate host ensure the antigens proteins receive normal eukaryotic structure and post-translational modifications. However, despite of intense research, much remains to be done to develop effective vaccine against parasites. Because it has been found that increased antigen expression correlates with improved immunogenicity in humans and large animals, next generation vectors should be adopted to improve antigen expression, manufacturing yield, quality, and regulatory compliance (Williams 2013). Further, selection of optimal protective antigens should be very careful, remembering that majority of parasites have co-evolved with their vertebrate hosts and have developed multiple strategies to persist asymptotically for the lifetime of the hosts. To enable this survival, these parasites have developed complex and multifaceted mechanisms to subvert or suppress host immunity.

References

Ahmad G., Torren W., Zhang W., Wyatt M., Siddiqui A.A. 2009a. Sm-p80-based DNA vaccine formulation induces potent protective immunity against Schistosoma mansoni. Parasite Immunology, 31, 156–161. DOI: 10.1111/j.1365-3024.2008.001091.x

Ahmad G., Zhang W., Torren W., Damian R.T., Chavez-Suarez M., Wolf R.F., White G.L. 2009b. Protective and antifecondity effects of Sm-p80-based DNA vaccine formulation against Schistosoma mansoni in a nonhuman primate model. Vaccine, 27, 2830–2837. DOI: 10.1016/j.vaccine.2009.02.096
Ahmad G., Zhang W., Torben W., Haskins C., Diggs S., Noor Z., Le L., Siddiqui A.A. 2009c Prime/boost and recombinant protein vaccination strategies using Sm-p80 protects against Schistosoma mansoni infection in the mouse model to levels previously attainable only by the irradiated cercarial vaccine. *Parasitology Research*, 105, 1767–1777. DOI: 10.1007/s00436-009-0646-z

Anand S.B., Murugan V., Prabhu P.R., Anandharaman V., Reddy M.V., Kaliraj P. 2011. A combination with plasmid DNA followed by recombinant vaccinia virus or gene gun delivery. *Parasitology Research*, 107, 106–112

Anand S.B., Kodumudi K.N., Prabhu P.R., Anandharaman V., Reddy M.V., Kaliraj P. 2011. Partial immunity to Schistosoma mansoni infection with plasmid DNA encoding invariant surface glycoprotein gene is able to induce partial protection in experimentally infected mice. *Journal of Helminthology*, 85, 442–452. DOI: org/10.1016/j.jhepm.2011.01.012

Bergman P.J., Camps-Palau M.A., McKnight J.A., Leibman N.F., Craft D.M., Leung C., Liao J., Riviere I., Sadelain M., Grenn-Schalhaus A.E., Gregor P., Houghton A.N., Perales M.A., Wolthoek J.D. 2006. Development of a xenogenic DNA vaccine program for canine malignant melanoma at the Animal Medical Center. *Journal of Parasitology*, 92, 1300–1303. DOI: 10.1086/500241

Bolhassani A., Gholami E., Zahedifar F., Doroudi M., Parsi P., Doustdari F., Seyed N., Papadopoulou B., Rafati S. 2011. Leishmania major: Protective capacity of DNA vaccine using amastin fused to HSV-VP22 and EGFP in BALB/c mice model. *Experimental Parasitology*, 128, 9–17. DOI: 10.1016/j.exppara.2011.01.012

Cao S., Mousa A.A., Aboge G.O., Zhou M., Moumouni P.F.A., Terkawi M.A., Masatani T., Nishikawa Y., Tada H., Webster D.P., Butcher G., Watkins K., Sinden R.E., Levine G.L., Rich P., Schneider J., Klosow D., Gilbert S.C., Carucci D.J., Hill A.V.S. 2006. A DNA Prime-Modified Vaccinia Virus Ankara Boost Vaccine Encoding Thrombospondin-Related Adhesion Protein but Not Circumsporozoite Protein Partially Protects Healthy Malaria–Naive Adults against Plasmodium falciparum Sporozoite Challenge. *Infection and Immunity*, 74, 5933–5942. DOI: 10.1128/IAI.00590-06

Espino A.M., Morales A., Delgado B., Rivero F.M., Figueroa O., Suárez E. 2010. Partial immunity to Fasciola hepatica in mice after vaccination with FlsAP2 delivered as recombinant protein or DNA construct. *Ethnicity & Disease*, 20, S1–27

Fukumoto F., Tamaki Y., Igarashi I., Suzuki H., Xuan X., 2009. Immunogenicity and growth inhibitory efficacy of the prime-boost immunization regime with DNA followed by recombinant vaccinia virus carrying the P29 gene of Babesia gibsoni in dogs. *Experimental Parasitology*, 123, 296–301. DOI: 10.1016/j.exppara.2009.08.012

Garver K.A., LaPatra S.E., Kurath G. 2005. Efficacy of an infectious hematopoietic necrosis virus (IHN) virus DNA vaccine in Chinook Oncorhynchus tschawytscha and sockeye O. niska salmon. *Diseases of Aquatic Organisms*, 64, 13–22. DOI: 10.3354/dao064013

Geriletu Xu L., Xurui Li X. 2011. Vaccination of chickens with DNA vaccine expressing Eimeria tenella MZ5-7 against coccidiosis. *Veterinary Parasitology*, 177, 6–12. DOI: 10.1016/j.vetpar.2010.11.041

Han K., Xu L., Yan R., Song X., Li X. 2012. Vaccination of goats with glycerolaldehyde-3-phosphate dehydrogenase DNA vaccine induced partial protection against Haemonchus contortus. *Veterinary Immunology and Immunopathology*, 149, 177–185. DOI: 10.1016/j.vetimm.2012.06.016. Epub 2012 Jun 19

He S., Yang L., Lv Z., Hu W., Cao J., Wei J., Sun X., Zheng H., Wu Z. 2010. Molecular and functional characterization of a mortality-like protein from Schistosoma japonicum (SMJP/ hsp70) as a member of the HSP70 family. *Parasitology Research*, 107, 955–966. DOI: 10.1007/s00436-010-1960-5

Heyzarijafari H.Z., Ghaffari Far F., Dalmi F., Sharifi F., Jorjani O. 2013. Effect of IL-22 on DNA vaccine encoding LACK gene of Leishmania major in BALB/c mice. *Experimental Parasitology*, 134, 341–348. DOI: org/10.1016/j.exppara.2013.03.012

Hiszczynska-Sawicka E., Olędzka G., Holec-Gajor L., Li H., Xua J.B., Sedcole R., Kuk J., Bickerstaffe R., Stankiewicz M. 2011a. Evaluation of immune responses in sheep induced by DNA immunization with genes encoding GRA1, GRA4, GRA6 and GRA7 antigens ofToxoplasma gondii. *Veterinary Parasitology*, 177, 281–289. DOI: 10.1016/j.vetpar.2010.11.047
Hiszczynska-Sawicka E., Li H., Xua J.B., Holec-Gasior L., Kur J., Sedcole R., Bickerstaffe R., Stankiewicz M. 2011b. Modulation of immune response to Toxoplasma gondii in sheep by immunization with a DNA vaccine encoding ROP1 antigen as a fusion protein with ovine CD154. Veterinary Parasitology, 183, 72–78. DOI: 10.1016/j.vetpar.2011.06.010

Januszkiewicz K. 2010. Optimization of vaccination against Fasciola hepatica infections using CDNA encoding for selected fluke antigens and specific for the host species CTLA-4 molecule. PhD Thesis. Witol Stefański Institute of Parasitology Polish Academy of Sciences, Warsaw, Poland

Jaros S., Jaros D., Wesolsowska A., Zygner W., Wędrychowicz H. 2010. Blocking Fasciola hepatica’s energy metabolism - a pilot study of vaccine potential of a novel gene – phosphoglycerate kinase. Veterinary Parasitology, 172, 229–237. DOI: 10.1016/j.vetpar.2010.05.008

Jayaraj R., Piedrafta D., Spithill T., Smooker P. 2012. Evaluation of the immune responses induced by four targeted DNA vaccines encoding the juvenile liver fluke antigen, cathepsin B in a mouse model. Genetic Vaccines and Therapy 2012, 10, 2–7, http://www.gvt-journal.com/content/10/1/7

Jenkins M., Parker C., Tuo W., Vinyard B., Dubey J.P. 2004. Inclusion of CpG adjuvant with plasmid DNA coding for NcGRA7 improves protection against congenital neosporosis. Infection and Immunity, 72, 1817–1819. DOI: 10.1128/IAI.72.3.1817–1819.2004

Kalyanasundaram R., Balumuri P. 2011. Multivalent vaccine formulation with BmVAL-1 and BmALT-2 confer significant protection against challenge infections with Brugia malayi in mice and jirds. Research and Reports in Tropical Medicine 2011, 45–56. DOI: 10.2147/RTRTMS13679

Kennedy N.J., Spithill T.W., Tennon J., Wood P.R., Piedrafta D. 2006. DNA vaccines in sheep: CTLA-4 mediated targeting and CpG motifs enhance immunogenicity in a DNA prime/protein boost strategy. Vaccine, 24, 970–979. DOI: 10.1016/j.vaccine.2005.08.076

Kimani D., Jagne Y.J., Cox M., Kimani E., Bliss C.M., Gitau, E., Ogwang C., Afolabi M.O., Bowyer G., Collins K.A. 2014. Translating the immunogenicity of prime-boost immunization with ChAd63 and MVA ME-TRAP from malaria naïve to malaria-endemic populations. Molecular Therapy, 22, 1992–2003. DOI: 10.1038/mt.2014.109.

Kliman D.M., Yamshchikov G., Ishigatsubo Y. Contribution of CpG motifs to the immunogenicity of DNA vaccines. Journal of Immunology, 158, 3635–3642

Kofa W., Wędrychowicz H. 2001. c-DNA vaccination against parasitic infections: advantages and disadvantages. Veterinary Parasitology 94; 243–247, DOI: 10.1016/S0304-4017(01)00478-2

Kurup S.P., Tewari A.K. 2012. Induction of protective immune response in mice by a DNA vaccine encoding Trypanosoma evansi beta tubulin gene. Veterinary Parasitology, 187, 9–16. DOI: 10.1016/j.vetpar.2012.01.009

Lee J.S., Kim I.S., Sohn W.M., Lee J., Yong T.S. 2006a. A DNA vaccine encoding a fatty acid binding protein of Clonorchis sinensis induces protective immune response in Sprague-Dawley rats. Scandinavian Journal of Immunology, 63, 169–176 DOI: 10.1111/j.1365-3083.2006.01721.x

Lee J., Kim I.S., Sohn W., Lee J., Yong T. 2006b Vaccination with DNA encoding cysteine proteinase confers protective immune response to rats infected with Clonorchis sinensis. Vaccine, 24, 2358–2366

Levine M.M. and Sztein M.B. 2004. Vaccine development strategies for improving immunization: The role of modern immunology. Nature Immunology, 5, 460–464. DOI: 10.1038/nri0504-460

Li M., Lei J., Wang T., Lu S., Guan F., Liu W., Li Y. 2011. Cimetidine enhances the protective effect of GST DNA vaccine against Schistosoma japonicum. Experimental Parasitology 128, 427–432. DOI: 10.1016/j.exppara.2011.05.012

Liddell S., Parker C., Vinyard B., Jenkins M., Dubey J.P. 2003. Immunization of mice with plasmid DNA coding for NcHSP33 confers partial protection against vertical transmission of Neospora caninum. Journal of Parasitology, 89, 496–500. DOI: org/10.1645/GE-2969

Liu, M.A. 2010. DNA vaccines: An historical perspective and view to the future. Immunological Reviews, 239, 62–84. DOI: org/10.1111/j.1600-065X.2010.00980.x

Liu Q., Shang L., Jin H., Wei T., Zhu Q., Gao H. 2010. The protective effect of a Toxoplasma gondii SAG1 plasmid DNA vaccine in mice is enhanced with IL-18. Research in Veterinary Science, 89, 93–97. DOI: 10.1016/j.rvsc.2010.01.007

Mancini-Bourings M., Fontaine H., Brechot C., Pol S., Michel M.L. 2006. Immunogenicity of a hepatitis B DNA vaccine administered to chronic HBV carriers. Vaccine, 24, 4482–4489. DOI: 10.1016/j.vaccine.2005.08.013

Mash S., Arora S.K., Vasištha R.K. 2011. Efficacy of Leishmania donovani ribosomal P1 gene asDNA vaccine in experimental visceral leishmaniasis. Experimental Parasitology, 129, 55–64. DOI: 10.1016/j.exppara.2011.05.014

Martin J.E., Louder M.K., Holman L.A., Gordon J.L., Enama M.E., Larkin B.D. et al. 2008 A SARS DNA vaccine induces neutralizing antibody and cellular immune responses in healthy adults in a Phase I clinical trial. Vaccine, 26, 6338–6343. DOI: 10.1016/j.vaccine.2008.09.026

McCullers J.A. 2007. Evolution, benefits, and shortcomings of vaccine management. Journal of Managed Care Pharmacy, 13, S2–S6

Mc Neilly T.N., Nisbet A.J. 2014. Immune modulation by helminth parasites of ruminants: implications for vaccine development and host immune competence. Parasite, 21, 51–62. DOI: 10.1051/parasite/2014051

Molloy M., Bouladoux N., Belkaïd Y. 2012. Intestinal microbiota: shaping local and systemic immune responses. Seminar in Immunology, 24, 51–55. DOI: 10.1016/j.smim.2011

Nielsen H.V., Di Cristina M., Bechetto E., Spadoni A., Peterson E., Garano N. 2006. Toxoplasma gondii: DNA vaccination with Bradyzoite antigens induces protective immunity in mice against oral infection with parasite cysts. Experimental Parasitology, 112, 274–279. DOI: 10.1016/j.exppara.2005.11.009

Payette P.J., Weeratna R.D., McCluskie M.J., Davis H.L. 2001. Immune-mediated destruction of transfected myocytes following DNA vaccination occurs via multiple mechanisms. Gene Therapy, 8, 1395–1400. DOI: org/10.1038/sj.gnt.3301534

Person R., Bodles-Brakhop A.M., Pope M.A., Khan A.S., Draghi-Akli R. 2008. Growth hormone-releasing hormone plasmid treatment by electroporation decreases offspring mortality over three pregnancies. Molecular Therapy, 16, 1891–1897. DOI: 10.1038/mt.2008.178

Qu D., Han J., Du A. 2013. Evaluation of protective effect of multi-antigenic DNA vaccine encoding MIC3 and ROP18 antigen segments of Toxoplasma gondii in mice. Experimental Parasitology Research, 112, 2593–2599. DOI: 10.1007/s00436-013-3425-0

Rafati S., Zahedifard F., Azari M.K., Taslimi Y., Taheri T. 2008. Leishmania infantum: Prime boost vaccination with C-terminal extension of cysteine proteinase type I displays both type 1 and 2 immune signatures in BALB/c mice. Experimental Parasitology, 118, 393–401. DOI: 10.1016/j.exppara.2007.10.004

Rainczuk A., Scorza T., Spithill T.W., Smooker P.M. 2004. A bistrionic DNA vaccine containing Apical Membrane Antigen 1 and Merozoite Surface Protein 4/5 can prime humoral and
cellular immune responses and partially protect mice against virulent Plasmodium chabaudi adami DS malaria. *Infection and Immunity*, 72, 5565–5573. DOI: 10.1128/IAI.72.10.5565-5573.2004

Rathaur S., Yadav M., Gupta S., Anandharaman V., Reddy M.V. 2008. Filarial glutathione-S-transferase: a potential vaccine candidate against lymphatic filariasis. *Vaccine*, 26, 4094–4100. DOI: 10.1016/j.vaccine.2008.03.099

Robinson M.W., Dalton J.P., O’Brien B.A., Donnelly S. 2013. *Fasciola hepatica*: The therapeutic potential of a worm secretome. *International Journal for Parasitology*, 43, 283–291. ttp://dx.doi.org/10.1016/j.ijpara.2012.11.004

Saade F., Petrovsky N. 2012. Technologies for enhanced efficacy of DNA vaccines. *Expert Review of Vaccines*, 11, 189–209. DOI: 10.1586/erv.11.188

Sato Y., Roman M., Tighe S., Naitza S., Hannan C.M., Aidoo M., Crisanti A., Robson K.J., et al. 2001. Genetic immunization induces partial protection against hookworm challenge infection. *Acta Parasitologica*, 58, 198–206. DOI: 10.1016/s0165-2427(03)00085-0

Shah M.A.A., Li X. 2010. A recombinant DNA vaccine encoding *Eimeria acervulina* cSZ-2 induces immunity against experimental *E. tenella* infection. *Veterinary Parasitology*, 169, 185–189. DOI: 10.1016/j.vetpar.2009.12.035

Sun W., Song X., Yan R., Li X. 2011. Vaccination of goats with *Fasciola hepatica* with cDNA encoding the future. *Veterinary Parasitology*, 156, 319–323. DOI: 10.1016/j.vetpar.2008.05.025

Van Riet E., Hartgers F.C., Yazdanbakhsh M. 2007. Chronic helminth infections induce immunomodulation: consequences and mechanisms. *Immunobiology*, 212, 6, 475–490. DOI: 10.1016/j.imbio.2007.03.009

Vaughan E.E., Dean D.A. 2006. Intracellular trafficking of plasmds during transfection is mediated by microtubules. *Molecular Therapy*, 13, 422–428. DOI: org/10.1016/j.ymthe.2005.10.004

Waine G.J., Alarcon J.B., McManus D.P. 2002. Genetic immunization of mice with DNA encoding the 23 kDa transmembrane surface protein of *Schistosoma japonicum* (Sj23) induces antigen-specific immunoglobulin G antibodies. *Parasite Immunology*, 1999, 21, 377–381

Wang H., He S., Yao Y., Cong H., Zha H., Li T., Zhu X. 2009. *Toxoplasma gondii*: Protective effect of an intranasal SAG1 and MIC4 DNA vaccine in mice. *Experimental Parasitology*, 122, 226–232. DOI: 10.1016/j.exp Paras.2009.04.002

Wahren W., Liu M.A. 2014. DNA vaccines: recent developments and the future. *Vaccines*, 2, 785–796. DOI: 10.3390/vaccines2040785

Wesołowska A., Norbury L., Januszukiewicz K., Jedlina L., Jaros S., Zawistowska-Deniakiz A., Zygner W., Wędrychowicz H. 2013. Evaluation of the immune response of male and female rats vaccinated with cDNA encoding a cysteine protease of *Fasciola hepatica* (FhPcW1). *Acta Parasiologica*, 58, 198–206. DOI: 10.2478/s11686-013-0120-3

Wędrychowicz H., Szymanski P., Pasiński L.J., Bienkowska-Szewczyk K. 2002. Humoral immune response of rats vaccinated with cDNA or protein form of glutathione S-transferase of *Fasciola hepatica* to infection with metacercariae of the fluke. *Helminthologia*, 39, 127–133

Wędrychowicz H., Lamparska M., Kęsik M., Kotomski G., Mieszczanek J., Jedlina-Pasińska L., Płucienniczak A. 2003. The immune response of rats to vaccination with cDNA or protein forms of the cysteine protease of *Fasciola hepatica*. *Veterinary Immunoimmunology and Immunopathology*, 94, 83–93. DOI: 10.1016/S0165-2427(03)00085-0

Williams J.A. 2013. Vector Design for Improved DNA Vaccine Efficacy, Safety and Production. *Vaccines*, 1, 225–249. DOI: 10.3390/vaccines1030225

Wiśniewski M., Jaros S., Bańska P., Capello M., Wędrychowicz H. 2013. *Ancylostoma ceylanicum* metalloprotease 6 DNA vaccine induces partial protection against hookworm challenge infection. *Acta Parasitologica* 58, 376–383. DOI: 10.2478/s11686-013-0151-9

Wolff J.A., Malone R.W., Williams P., Chong W., Acsadi G., Jani A., Felgner P.L. 1990. Direct gene transfer into mouse muscle by a multiantigenic SAG1-ROP2 DNA vaccine. *Immunobiology*, 2, 785–796. DOI: org/10.1016/j.vetpar.2012.11.006
Antiparasitic DNA vaccines

Experimental Parasitology, 119, 352–357, DOI: 10.1016/j.exppara.2008.03.005

Xue M., He S., Zhang J., Cui Y., Yao Y., Wang H. 2008b. Evaluation of the immune response elicited by multi-antigenic DNA vaccine expressing SAG1, ROP2 and GRA2 against Toxoplasma gondii. Parasitology International 57, 424–429. DOI: 10.1016/j.parint.2008.05.001

Yamakami K., Akao S., Sato M., Nitta Y., Miyazaki J., Tadakuma T. 2001. A single intradermal administration of soluble leishmanial antigen and plasmid expressing interleukin-12 protects BALB c mice from Leishmania major infection. Parasitology International, 50, 81–91

Yan H., Yuan Z., Petersen E., Zhang X., Zhou D., Liu Q., He Y., Lin R., Xu M., Chen X., Zhong X., Zhu X. 2011. Toxoplasma gondii: Protective immunity against experimental toxoplasmosis induced by a DNA vaccine encoding the perforin-like protein 1. Experimental Parasitology, 128, 38–43. DOI: 10.1016/j.exppara.2011.02.005

Yan R., Sun W., Song X., Li X. 2013. Vaccination of goats with DNA vaccine encoding Dim-1 induced partial protection against Haemonchus contortus: A preliminary experimental study. Research in Veterinary Science, 95, 189–199. DOI: 10.1016/j.rvsc.2013.02.020

You Z., Huang X., Hester J., Toh H.C., Chen S.Y. 2001. Targeting dendritic cells to enhance DNA vaccine potency. Cancer Research, 61, 3704-3711

Yu Q., Li J., Zhang X., Gong P., Zhang P., Li S., Wang H. 2010. Induction of immune responses in mice by a DNA vaccine encoding Cryptosporidium parvum Cp12 and Cp21 and its effect against homologous oocyst challenge. Veterinary Parasitology: 172, 1–7. DOI: 10.1016/j.vetpar.2010.04.036

Yu L., Yamagishi J., Zhang S., Jin Ch., Aboge O.G., Zhang H., Zhang G., Tanaka T., Fujisaki K., Nishikawa Y., Xuan X. 2012. Protective effect of a prime-boost strategy with plasmid DNA followed by recombinant adenovirus expressing TgAMA1 as vaccines against Toxoplasma gondii infection in mice. Experimental Parasitology, 61, 481–486. DOI: 10.1016/j.parint.2012.04.001

Yuan H., You-en S., Long-jiang Y., Xiao-hua Z., Liu-zhe L., Cash M., Lu Z., Zhi L., Deng-xin S. 2006. Studies on the protective immunity of Schistosoma japonicum bivalent DNA vaccine encoding Sj23 and Sj14. Experimental Parasitology, 115, 379–386. DOI: 10.1016/j.exppara.2006.09.022

Zhao Y., Bao Y., Zhang L., Chang L., Jiang L., Liu Y., Zhang L., Qin J. 2013. Biosafety of the plasmid pcDNA3-IE of Eimeria acervulina in chicken. Experimental Parasitology, 133, 231–236. DOI: 10.1016/j.exppara.2012.11.026

Zhao G., Yan R., Muleke C.I., Sun Y., Xu L., Li X. 2012. Vaccination of goats with DNA vaccines encoding H11 and IL-2 induces partial protection against Haemonchus contortus infection. Veterinary Journal, 191, 94–100. DOI: 10.1016/j.tvjl.2010.12.023. Epub 2011 Feb 16.

Zhu Y., Lu F., Dai Y., Wang X., Tang J., Zhao S., Zhang Ch., Zhang H., Lu S., Wang S. 2010. Synergistic enhancement of immunogenicity and protection in mice against Schistosoma japonicum with codon optimization and electroporation delivery of SJTPI DNA vaccines. Vaccine, 28, 5347–5355. DOI: 10.1016/j.vaccine.2010.05.017

Zhu L., Liu H., Lu M., Long Q., Shi Y., Yu L. 2011. Construction, purification, and evaluation of multivalent DNA vaccine against Schistosoma japonicum Parasitology Research, 108, 115–121. DOI: 10.1007/s00436-010-2040-6

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