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INVITED REVIEW

Antiparasitic DNA vaccines in 21st century

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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 immunostimulatory 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 Wollff'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-

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tion 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 intramuscularily 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 polycistronic 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-C-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-Cp21 groups. The reduction in the level of oocysts shedding from the group of pVAX1-C-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 (Hiszczyńska-Sawicka *et al.* 2011 a,b). Upon mixing with cationic liposomes, plasmid DNA is condensed into lipoplexes 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 initialy 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 studes 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)

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Table I. Selected DNA vaccination trials against vector transmitted protozoa in years 2001-2014

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 proteinase 1	prime/boost	no protection	Rafati *et al.* 2008
L. major	mouse	SLA+ IL12	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. yoeli	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

Table II. Selected DNA vaccination trials against food/water transmitted protozoa in years 2001-2014

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	NcSAG1- and NcSRS2	prime/boost	effective protection	Cannas *et al.* 2003
N. caninum	mouse	NcGRA7 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	Hiszczyńska-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

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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	5401in *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.	crossprotection against *E. tenella*	Shah *et al*. 2010
E. tenella	14 days	MZ5-7 +chicken IL-17	i.m.	partial protection to *E. tenella* challenge	Geriletu *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

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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.	im injection induced mixed Th1/Th2 response	Smooker *et al.* 2001
F. hepatica	mouse	CatL	i.m.	predominantly Th2 related respone (IgG1)	Smooker *et al.* 2001
F. hepatica	rat	GST	i.n.	decreased worm burden by 54%, IgG2b	Wedrychowicz *et al.* 2002
F. hepatica	rat	CatL	i.m.	decreased worm burden by 61–75%, Th2 response	Wedrychowicz *et al.* 2003
F. hepatica	sheep	FhCatB+CTLA4	i.m.	enhanced immune response following vaccination	Kennedy e al. 2006
F. hepatica	sheep	FhPGK+CTLA4	i.m.	better weight gain after challenge infection	Januszkiewicz 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

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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; Wesołowska *et al.* 2013).

Why it is 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 (Wędrychowicz *et al.* 2003; Wesołowska *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 verterbrate host ensures that antigenic 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 asymptomatically for the lifetime of the hosts. To enable this survival, these parasites have developed complex and multifaceted mechanisms to subvert or suppress host immunity.

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