DNA Vaccines against Viral Diseases: Basic Immunological Aspects and Applied Perspectives

Niels Lorenzen*, Ellen Lorenzen, Jesper Skou Rasmussen and Katja Einer-Jensen

National Veterinary Institute, Technical University of Denmark, Hangevej 2,
DK-8200 Aarhus N, Denmark

DNA vaccination is based on the administration of the gene encoding the vaccine antigen, rather than the antigen itself. Subsequent expression of the antigen by transfected cells in the vaccinated host triggers the host immune system. Among the many experimental DNA vaccines tested in various animal species as well as in humans, the vaccines against diseases caused by rhabdoviruses in fish have given some of the most promising results (Kurath 2008; Kurath *et al.*, 2007; Lorenzen and LaPatra, 2005).

A single intramuscular injection of micro-gram amounts of a DNA vaccine encoding the viral glycoprotein(G) induces rapid and long-lasting protection against important viruses such as IHNV and VHSV in rainbow trout *Oncorhynchus mykiss*. Immunity is established already a few days after vaccination. Cross-challenge experiments have revealed that protection in the early phase is mediated by non-specific antiviral mechanisms. Within 2–4 weeks at 12–15°C, the protection changes into a specific phase in which the fish are immune only to the virus species homologous to the vaccine (LaPatra *et al.*, 2001; Lorenzen *et al.*, 2002a, b). The immunity does cover within-species sero- and genotype variations, but the level of protection is highest against the homologous variant (Lorenzen *et al.*, 1999; Lorenzen *et al.*, 2000). By the use of an IFN type I inducible Mx-promoter reporter gene cell line, it has been demonstrated that transfected cells expressing the VHSV G protein can induce IFN production in neighbouring cells (Acosta *et al.*, 2006). Correspondingly, a systemic upregulation of Mx gene expression is detectable *in vivo* shortly after DNA vaccination and timely correlates with early protection (McLauchlan *et al.*, 2003). The early protection is therefore most likely due to induction of IFN-activated defence mechanisms. In the long-lasting specific immunity phase, both virus-neutralizing antibodies and cytotoxic cells specific for virus-infected target cells can be detected, the latter presumably including both cytotoxic T-cells and Natural

Killer cells (Boudinot *et al.*, 1998; Utke *et al.*, 2008). The dual-phase immune activation pattern is comparable to the typical immune response kinetics known from higher vertebrates mounting a protective immune response to a pathogen infection: immediate innate mechanisms are rapidly activated and keep the infection under control, giving the organism time to develop an adaptive immune response, which, in most cases, efficiently eliminates the pathogen (Fig. 1).

Time-course immunohistochemical analysis of the site of DNA injection has revealed that a high vaccine dose of approx. 20 µg plasmid DNA encoding the VHSV G protein induces an inflammatory response with a transient massive infiltration of leucocytes mediating degradation of muscle cells expressing the viral glycoprotein. The local inflammatory response is most likely induced by the mature G protein appearing on the surface of transfected cells and hereby triggering IFN type I and other innate mechanisms, as discussed above. Transfected cells would further be expected to present G peptides in MHC class I context as a result of

Fig. 1. The diagram illustrates how the protection induced by DNA vaccination against VHS and IHNV in rainbow trout is a combination of rapidly activated general anti-viral mechanisms followed by more slowly induced specific and long-lasting immunity. Based on Lorenzen *et al.* (2002b).

* Corresponding author
E-mail: nilo@vet.dtu.dk

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Fig. 2. **a:** Schematic illustration of expression of the VHSV G protein in cells transfected with a DNA vaccine construct encoding the VHSV G gene. In the cell nucleus, the gene is transcribed into mRNA, which is exported to the cytoplasm where ribosomes translate the gene into a polypeptide. The first 21 amino acids constitute the signal peptide, making the ribosomes attach to the endoplasmic reticulum (ER) and guiding the growing polypeptide into the ER lumen and later Golgi compartments, where processing, glycosylation and folding takes place before the mature transmembrane protein finally appears as a trimeric molecule on the cell surface. This makes the transfected cell appear like a virus infected cell and activates a strong inflammatory response, as seen in **b**. **b:** Transverse section of the muscle injection site (27 days post vaccination), stained immunohistochemically for G protein. The infiltrating cells attach, kill, phagocytose and finally eliminate the transfected muscle cells. (Based on Lorenzen *et al.*, 2005).

endogenous expression of the G protein (Rock and Goldberg 1999). Plasmid in the cell nucleus is missing. Please see the original submission. Presence of antigen positive macrophage-like cells, presumably due to phagocytosis of G protein expressed by muscle cells, suggests that also MHC class II restricted presentation of antigen peptides may occur (Lorenzen *et al.*, 2005, Fig. 2). In this way, the expressed G protein is presumably able to activate both the innate and the two adaptive arms of the immune response.

Some functional studies related to practical use of the DNA vaccines against VHS and IHNV have been made. Activation and maintenance of the innate and adaptive immune response mechanisms, respectively, most likely depend on different properties of the viral G protein, and are temperature dependent in a complementary way: At low temperatures, *i.e.* approx. 5°C, the innate cross-protective antiviral mechanisms last more than two months, while generation of specific neutralizing antibodies appears suppressed. At higher temperatures (in the case of rainbow trout, approx. 15°C), the innate cross protective period lasts a few weeks and neutralizing antibodies are often detectable shortly after. While simultaneous DNA vaccination against VHSV and IHNV induced good protection against both viruses, simultaneous intraperitoneal injection of an oil-adjuvanted bacterial vaccine compromised the early protective effect of the VHSV DNA vaccine (Einer-Jensen *et al.*, 2009, *in press*, and authors' unpublished observations).

A few field studies have also been performed. A preliminary testing of the VHSV DNA vaccine under field conditions was recently done in Denmark. The fish

were vaccinated during the winter at farms without disease and were then transferred to net-pens on farms with ongoing VHS outbreaks during spring. A high cage-to-cage variability was found and other diseases than VHS occurred in several pens. However, on an overall basis, the results supported that DNA vaccination against VHS has an applied potential. Safety is high in terms of animal health, since no pathogen is present in the vaccine and no significant side effects have been seen. In British Columbia, Canada, a commercial DNA vaccine against IHNV in Atlantic salmon *Salmo salar* became available in 2005 (Salonius *et al.*, 2007). This represents a breakthrough in the veterinary use of DNA vaccines for husbandry animals, and it may be expected that other veterinary DNA vaccines will follow.

Table 1. Comparison of the protective effects of homologous (VHS) and heterologous (IHNV) DNA vaccines against challenge with VHSV at different times post vaccination. Based on Lorenzen *et al.* (2002a).

Vaccine	Time of challenge ^a			
	4 dpv	7 dpv	60 dpv	84 dpv
Plasmid vector	96% ^b	95%	91%	96%
pVHS-G	21%*	19%*	1%*	5%*
p IHNV-G	41%*	12%*	94%	96%
Unhandled	96%	91%	86%	99%

^a Rainbow trout fingerlings (3 g average weight) were vaccinated with 1 µg DNA at day 0 and subgroups were bath-challenged with VHSV in triplicate aquaria at different days post vaccination (dpv).

^b Values are the cumulated percentages of mortality 21 days after challenge.

* indicates significant protection compared to unhandled fish.

However, despite several attempts to develop efficiently working DNA vaccines to other fish pathogens, the results have not been as robust and reproducible as in the case of the rhabdoviruses (Kurath *et al.*, 2008; Lorenzen and LaPatra, 2005). A future research challenge is therefore to dissect the immune response mechanisms induced by the rhabdovirus DNA vaccines at a molecular level in order to identify crucial elements which may be of general use.

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