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DNA Vaccine: Mechanism of Action and Factors which Increase Its Efficacy

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A B S T R A C T

DNA vaccine is a new promising vaccine type which has many advantages among various conventional vaccines. DNA vaccine could induce the cellular immune response beside the humoral immune response. This vaccine is more stable in production, storage and distribution, which makes it as a cheaper vaccine. Although it has many added values, so far there is no FDA accepted human vaccine. There are some hindering factors that affect the efficacy of the DNA vaccine, such as immunogenicity, delivery system, administration route, and adjuvant choices. This review aims to describe the DNA vaccine mechanism of action and several factors that have important roles to increase the DNA vaccine efficacy. By optimizing these factors, an effective DNA vaccine could be developed.

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Introduction

Vaccination is one of the most successful immunology application to increase human health [1]. Since its early development several hundred years ago, vaccination has been developed in several types, including conventional vaccine which contains inactivated pathogen, subunit vaccine, and toxoid vaccine. One of the most prominent new vaccines is DNA vaccine. Although it has been developed for almost 20 years, DNA vaccine still cannot outperform its previous, such as therapeutics protein vaccine or inactivated pathogen vaccine [2]. This is an unfortunate condition since DNA vaccine has superiorities compare to other vaccine types.

In bigger scale, DNA vaccine is easier to produce than the protein-based vaccine, therefore the production cost can be minimized so that the price is lower than the conventional vaccine. Moreover, manipulation in the DNA level reduces the pathogen contamination and increases the vaccine safety. Additionally, protein express inside the host cell has similar conformation with the native antigen so it will have greater potency. DNA vaccine also triggers the antigen expression presented by type I or II Major Histocompatibility Complex (MHC) to stimulate cellular and humoral immune response [2, 3].

DNA vaccines comprise plasmids containing genes or parts of antigenic genes expressed in host cells. The expressed antigen will induce host immune system. To be expressed in the host cell (eukaryotic system); the DNA vaccine is also equipped with an expression system recognized by the eukaryotic system, in this case, the human expression system as the target of vaccination [2]. Components that are required in the DNA plasmids as vaccine carriers are eukaryotic promoters, multiple cloning sites, polyadenylation sequences, marker selection and bacterial origin of replication (Ori).

Strong promoters are needed so that the antigen gene can be recognized by eukaryotic cell expression systems, cytomegalovirus (CMV) or simian virus 40 (SV40) promoters are commonly used [4, 5]. Multiple cloning sites, located downstream to the promoters, are used to insert the antigen gene into the plasmids. Polyadenylation sequences are used to stabilize the mRNA, usually a bovine growth hormone (BGH) poly A sequence or SV40poly A are chosen. Selection markers are the antibiotic resistance encoding genes, such as ampicillin or kanamycin resistant coding genes. Ori is used in the plasmid replication process by host bacteria, such as *Escherichia coli* as the most popular bacteria for the production of DNA vaccine [6].

In this review, the mechanism of DNA vaccine to induce immune system and several factors which related to its efficacy are discussed.

DNA vaccine mechanism of action in triggering antigen presentation

Once the DNA vaccine enters the cell, the antigen is expressed in small amounts, picogram to nanogram scale via host cell expression system (human). Therefore, the antigen should be able to trigger the immune system, which begins with presentation by antigen presenting cells (APC). There are three studied mechanisms for DNA vaccine antigen processing and presenting, i.e. priming somatic cells (keratinocytes and myocytes), direct transfection by APC, and cross-priming [7].

The first suggested mechanism is that the somatic cells play directly as APC. DNA vaccines are generally administered by intramuscular injection directly so that DNA enters the smooth muscle cells, however smooth muscle cells cannot properly present antigen through Class I MHC pathways. The study showed that although smooth muscle cells play role in immune system induction, the mechanism should be supported by other cells, in this case, professional APC. In addition to intramuscular injection, DNA vaccine is also administrated through sub-cutaneous and targeted to keratinocyte cells. Although studies showed that the keratinocyte cells is needed absolutely in immune system induction, keratinocyte cells alone cannot present antigen well. The second mechanism assumes that the injected DNA is directly transfected or entered a professional APC cell, such as dendritic cells. Studies showed that DNA vaccines undergoing transfection into dendritic cells are only a small

number, but have a crucial role in the process of presenting antigens. The third mechanism is cross-priming, where the DNA vaccines undergo transfection into somatic cells and then be phagocytized by APC, which process and present the antigen. The third mechanism is thought to be the most explaining mechanism how the antigen from DNA vaccine can initiate the immune system [2, 7].

Intracellular DNA sensing mechanism

Activation of the immune system by DNA begins with interferon and/or cytokine expression initiation. DNA induces the interferon expression in dendritic cells, macrophages, and fibroblasts. Immune activation starts with the entry of double-stranded DNA into the cell cytosol via Fc IIa receptors or scavenger receptors, e.g. CXCL16. Inside the cell, the DNA is captured by several other receptors, such as DLM-1 / ZBP-1 (also called DNA-dependent activator / DAI), Toll Like Receptor 9 (TLR 9) that presents on the surface of the endosome [5], AIM2-like receptor IFI16, or helicase DDX41 [8]. TLR 9 is expressed in dendritic cells.B cells are the only receptors of bacterial DNA and synthetic nucleotides with CpG motifs that can enhance the immune response [3]. TLR 9 interacts with surface receptors and other intracellular proteins to recognize and distinguish foreign DNA from DNA present in cells [5].
TLR9 and MyD88 recognize the DNA vaccine. TLR9 and Myd88 further form a complex along with IRF5, IRAK4, and TRAF6. This complex activates the NF-kB transcription factor through the Ikb kinase complex and MAP-kinase via Ubc13 and TAK1.

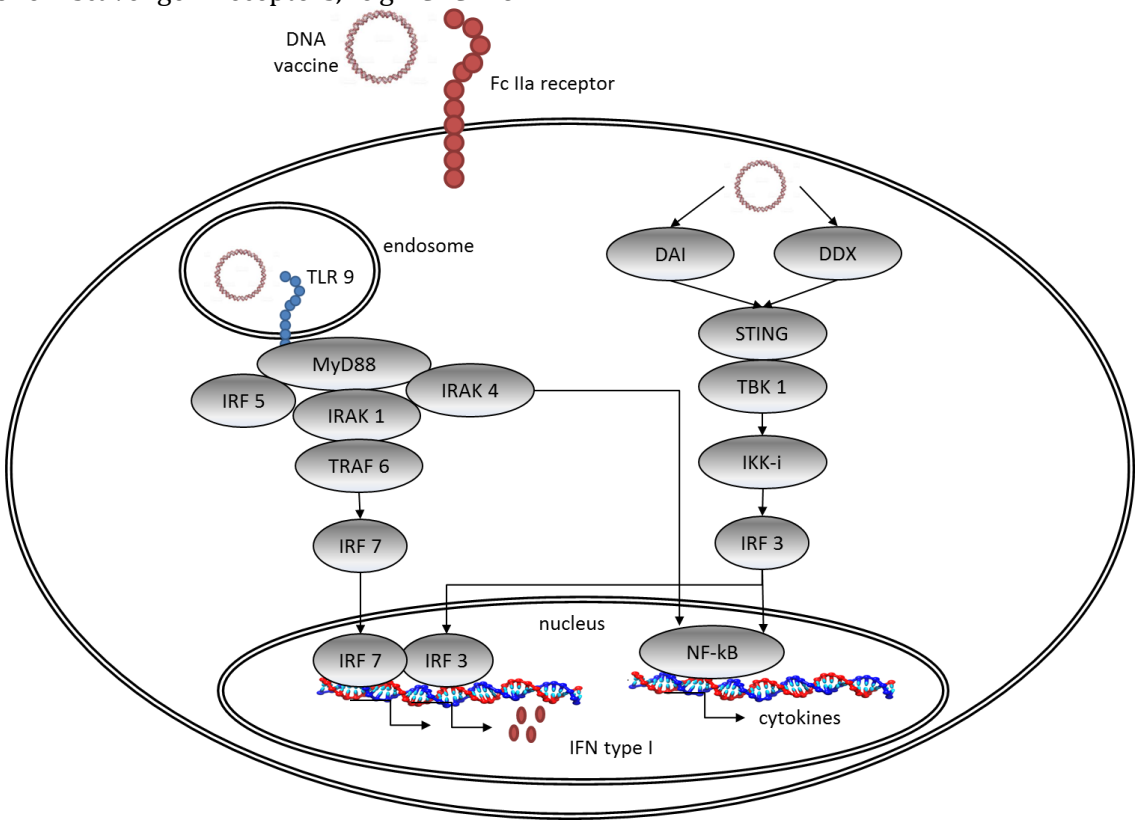

Fig. 1. Intracellular DNA signaling

The transcription factor increases the cytokines and chemokines expression. In addition, the toll / IL-1R domain-containing interferon-beta inducing adapter (TRIF) plays a role in the TLR-3 and TLR-4 signaling processes that induce interferon gene expression through TBK1. TBK1 induces IKK-i or

also known as K-IKK-Σ which further phosphorylates IRF3 and IRF7 that regulate IFNα and IFNβ expression [3, 5, 9]. In addition, stimulators of interferon genes (STING), localized in the endoplasmic reticulum, are also known to activate NF-kB and IRF3. STING stimulates IFN type I production after stimulated by dsDNA [10]. Induction of IFN type I production is also moderated by TRAF-family-member-associated NFKB activator (TANK) binding kinase 1 (TBK-1) - interferon regulatory factor 3 (IRF-3) (Figure 1).

IFN type I (IFN α and β) is captured by IFNα / β receptors expressed on the surface of almost all cell types. Furthermore, within the cells, IFN activates the Janus Kinase (JAK) I and II pathways and induces the transducer and activator of transcription (STAT) signals. This activation complex enters the nucleus and induces IFN-stimulated response elements (ISREs) which further regulates the anti-bacterial and anti-viral activity genes transcription. In addition, other gene regulations that are also affected by this complex are genes that participate in pro-inflammatory and anti-inflammatory production (e.g. interleukin-10) and cell apoptosis [11]., IFN type I also plays roles in T helper 1 (Th1) response induction, cross-presentation antigen to CD8 + T cells, and stimulation of CD8 + T cell proliferation. Type I IFN also stimulates antibodies production and isotype B cell switching [12].

DNA vaccine mechanism of inducing cellular and humoral immune response

An antigenic gene in the DNA plasmid is expressed using a host (human) expression system. This antigen is presented by APC through MHC class I (for endogenous antigens) or MHC class II (for exogenous antigens). APCs containing antigens drains into the lymph nodes through afferent lymphatic vessels. In the lymph nodes, the antigen is presented to the naïve T cells and T cell receptor (TCR) along with co-stimulatory molecule, resulting in the immune responses initiation and T cells expansion. T cells response through T helper cells CD4+ activation. This activation stimulates the cytokines production that plays roles in the B cells and cytotoxic T cells activation. CD4+ T cells can differentiate into various types of T helper cells, depending on the cytokines, such as IL-6 and IL-21 triggers the Th17 cells formation while IL-2 and IL-4 promote the Th2 cells formation. In addition, the antigen is also captured by the B cell receptor then it is processed and presented to CD4 T helper cells. Later on, the T helper cells facilitate an effective B cell response with the help of IL-21. Cytotoxic T lymphocytes CD8 + (CD8+ CTLs) are activated when the antigen endogenously presented by MHC class I. Activated T cells and B cells are returned to circulation via the lymphatic vessels efferent and provide defense against antigens [12–14]. The immune system induction by the DNA vaccine is presented in Figure 2.

Activated CD8 + CTLs react to antigen in peripheral tissues. CTLs bind to their antigens through complex bonds that is mediated by antigen receptors, co-receptors (CD8), and adhesion molecules (e.g. ICAM-1). The bond is very specific, thus it only recognizes cells that express the target antigen. It is formed between CTLs and target cells in an area which is called synapse. Furthermore, CTLs secrete granules (granzymes and perforins) proteins that induce target cell apoptosis. Perforin polymerizes and forms pores on the target cell membrane. Granzyme cuts substrates in cells and induces caspase activation. CTLs also induce cell apoptosis without granule secretion, but through Fas-ligand (FasL) secretions that induce extracellular apoptosis mechanism through its association with Fas death receptor. After delivering the granules and FasL, CTLs discharge from the target cells. CTLS also expresses cathepsin B, a protein that plays a role in the CTLs protection against perforin [15].

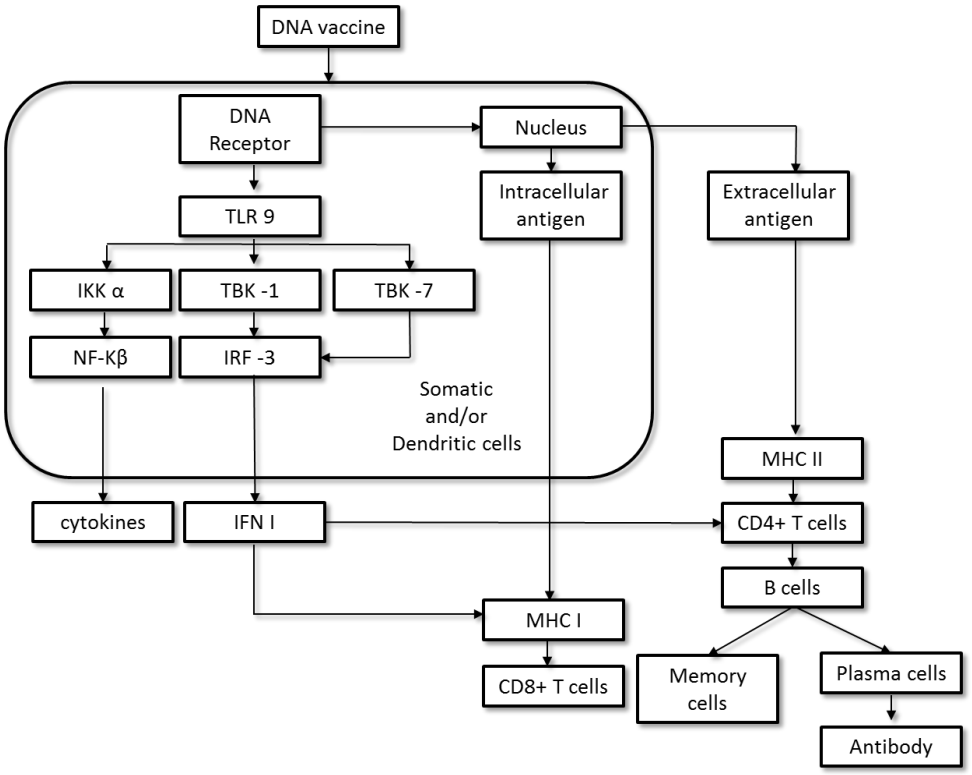

Fig. 2. DNA vaccine mechanism in inducing immune system.

Apoptotic cells release apoptotic cell-associated molecular patterns (ACAMPs) or damage-associated molecular patterns (DAMPs) that are being recognized by pattern-recognition receptors (PRRs) expressed by macrophage cells. Although they have similar structures with pathogen-associated molecular patterns (PAMPs), ACAMPs induce different macrophage responses. The PAMPs presentation is followed by a pro-inflammatory response, whereas the ACAMPs presentation does not induce a pro-inflammatory response. In addition, ACAMPs induce a response that is TLR-independent while PAMPs response is TLR-dependent. After the apoptotic molecules recognition, the next step is the binding of apoptosis cells with various receptors on the macrophage cell surface, e.g. lectin, avb3, CD36, SR-A, MER, CD14, ABCA1, PSR, CR3, CR4, CD91 /calreticulin, CD31, FccR, SHPS-1, or oxidation-specific receptors. The binding process is followed by the signaling process within the macrophage cells that induces cytokine production, initiation of the engulfment process, and phagocytosis [5, 16, 17].

B cell receptors on the B cells surface capture the antigens expressed as extracellular proteins. B cells require CD4 + T cells for the activation process. T cells helper expresses CD40L, which plays role in the cytokines secretion, such as IFN. Type I CD40L and IFN binding stimulates cell proliferation and differentiation. With the help of cytokines and follicular helper T cells (TFH), the affinity maturation of B cell affinity occurs in the germinal center [12]. Affinity maturation occurs through a somatic mutation process in IgG heavy chain and light chain. The result of this affinity maturation is B cells that can produce antibodies with high affinity. In addition, with the T cells helper assistance, isotype switching process occurs through recombination switching, this process leads to various Ig isotope production [15].
B cells differentiate into plasma cells and memory cells. Plasma cells migrate to the spinal cord while the memory cells circulate between the lymph nodes and the spleen, and respond rapidly when encountering antigens in the circulation. The humoral immune response is primarily mediated by antibodies produced by plasma cells. The

antibody-mediated reactions include neutralization of the antigen, opsonization assistance and antigen phagocytosis, complement activation, and cellular cytotoxic induction. The neutralization process of toxins and antigens is completed by blocking antigen or toxin binding with cells. IgG facilitate the opsonization through the antigen binding to the Fc receptors (FcγRI) that are presented on the phagocyte surface. Opsonized particles is internalized in vesicles called phagosomes and then undergo fusion with lysosomes. Furthermore, the antigens are degraded within phagolysosomes [15].

CpG motif as immune system inducer

The bacterial plasmids used in DNA vaccines contain unmethylated CpG motifs that trigger the human immune system [3]. CpG motif is recognized as the pathogen-associated molecular pattern (PAMP) [2] and induces the immune system through its binding with the Toll-Like Receptor 9 (TLR 9). The CpG motif is 20 times more common in bacteria than in higher vertebrates [18] and often referred as immunostimulatory DNA [9, 19]. However, unmethylated CpG motifs also present in the mammal's genome, but it does not be recognized by the TLR9, suggesting that the frequency of the motifs might have roles in the TLR9 stimulation. CpG motif also increases the production and secretion of cytokines and chemokines through Nf-kB activation [4, 19].

There are 4 types of CpG that have been identified which has a different structure and immunological activity [19, 20] (Table 1).

Table 1. CpG motif type and its activities.

No	CpG type	Activity
1	D	Stimulates pDC maturation and secretion of IFN-α
2	K / B type	Induces maturation and proliferation of B cells; triggers pDC differentiation and production of cytokines (IL-12p70, Il-6, and TNF-α)
3	C	Stimulates secretion of IL-6 from B cells and IFNα from pDC
4	P	Induces production of IFN type I

DNA vaccine delivery

Although the naked vaccine DNA has been studied to successfully deliver the antigen gene to the host cells [21, 22], there are several attempts to increase its efficacy. DNA vaccine has weakness in the in its low amount of expression *in vivo*, thus it cannot trigger immune system efficiently. Therefore, a delivery system that targets the DNA vaccine to the immune system is required. Several delivery systems that have been developed to deliver DNA vaccines is to use lipid carrier (liposome), nanoparticles [23, 24], and the bacterial live vector [25–27].

The use of microorganisms (live bacteria) as a vector or carrier of the vaccine was developed because the bacteria have special characteristics, such as lipopolysaccharide (LPS) in Gram-negative bacteria, lipoteicoid acid (LTA) in the Gram-positive bacteria, or PAMP which are recognized by PRR. All of them can trigger the immune system effectively. The immune response induced by live attenuated bacteria stimulates the long-term adaptive immune system. Thus it is suitable for use as a vaccine vector [27]. Moreover, the bacterial live vector provides a chance of a new delivery route, i.e. mucosal route, which is a promising alternative route to other invasive routes. The bacterial live vector can induce mucosal immune system as well as humoral and/ or cellular immunity. This ability is very advantageous since the mucosal route is often becoming a first gate of the pathogen entry [25].

DNA vaccines Delivery using live bacteria gives other several advantages, such as it is not requiring DNA purification process, but through the fermentation process of bacterial culture. Moreover, dendritic cells (DC) or other APC directly recognizes the bacteria. In the DC, the bacteria also act as an adjuvant because it has PAMP which initiating the innate immune system that leads activation of the adaptive immune

system [27]. Several types of bacteria that have developed as vaccine carriers are *Salmonella typhi and S. typhimurium, Listeria monocytogenes, Bordetella pertussis, Streptococcus gordonii, Vibrio cholerae, Mycobacterium bovis (BCG), Yersinia enterocolitica,* and *Shigella flexnery* [25–29].

Another bacteria that has been studied to deliver DNA efficiently is the lactic bacteria. The lactic bacteria have several advantages to deliver DNA vaccine since it has been used for ages in food fermentation process and generally recognized as safe (GRAS) bacteria. It is also resistant to the acid condition in the gastrointestinal. Additionally, the lactic bacteria has the ability to attach to the surface of the mucosal epithelial, therefore increase the retention time in the gastrointestinal tract. Some strains also have adjuvant properties which can induce higher immune response [30].

Lactococcus lactis is the most studied lactic bacteria since its genome was sequenced first, it is easily manipulated and engineered [31, 32]. Native *L. lactis* has been proven successfully deliver DNA to the epithelial cell *in vitro* [33–36] and *in vivo* [35, 37]. Some other studies used recombinant *L. lactis* combined with the *Staplycoccus aureus* Fibronectin Binding Protein A or mutated *Listeria monocytogenes* Internalin A. Both of them were proven more invasive than the wild-type *L. lactis* and delivered the DNA plasmid to the epithelial Caco-2 cells efficiently [34, 38].

Several other strains of the lactic bacteria that have been studied to deliver DNA vaccine are *Lactococcus casei, Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus bulgaricus, Lactobacillus salivarius, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus helveticus,* and *Lactobacillus gasseri* [30, 32, 39].

DNA vaccine adjuvant

To increase the vaccine efficacy, it is a compulsory to add adjuvant in the vaccine formulation. The adjuvant is needed to help increase, maintain, and directing the antigen to the immune response. Adjuvant also has roles in modulation the right immune response, minimize the administrated antigen, and increase vaccine efficacy, especially in the newborn, pediatric, and immune-compromised patients [40]. There are many types of adjuvants with the different mechanism of actions. A brief explanation of the several adjuvants of DNA vaccine is described in Table 2.

Table 2. Adjuvant types and its mechanism of action

No	Adjuvant types	Mechanism of action	Reference
1.	Mineral salts (aluminum oxyhydroxide and aluminum hydroxyphosphate)	Depot effect (maintain the antigen in the administration site), APC activation, antibody response stimulation, increase the pro-inflammation mediators releasing	[41, 42]
2.	Monophosphoryl lipid (MPL)	Immunostimulatory activity through macrophages activation and cytokines production stimulation	[43]
3.	Emulsi (e.g. Freund's incomplete adjuvant)	Depot effect in the administration site, increase the antigen releasing profile, stimulate plasma cells in antibody production	[43]
4.	Subunit B cholera toxin	Mucosal immune system induction through IgA production.	[40]
5.	Virus like particle (VLP)	As antigen repeated structure mimics virus capsid which form cross link with the B cell receptor, thus increase the high titer of IgG.	[43]

Concluding remarks

Although preclinical DNA vaccine trials have yielded promising results, clinical trials in humans generally show low effectiveness. This is caused by several factors, including the immune response which differs between species, the minimal levels of expressed antigens, and the DNA delivery systems. To anticipate this problem many methods have been developed, for example through CpG motifs modification to trigger the human immune system, the development of better

delivery systems, as well as the usage of various types of adjuvant to enhance the immune response. One of the unique methods that have been used in improving the effectiveness of DNA vaccine is by using live bacteria as vectors as a "vehicle" to deliver the DNA vaccine into the human body.

Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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