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A New Potent Route of DNA Vaccine Inoculation: DNA-Liposome Complexes on Bare Skin Induce Antigen-Special Antibody Responses

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Abstract: Transcutaneous immunization is a novel strategy for genetic vaccine immunization to induce detectable antigen-special antibody in humor and mucosal. In this study, plasmid expressing hepatitis B surface antigen (pGFP-HBsAg) was encapsulated in liposome, then DNA-liposome complexes were glued on bare skin of mice ear in different dosage (50μg, 10μg and 1μg). As control, DNA-liposome complexes of pGFP-HBsAg and pGFP vector were inoculated intraperitoneally. The anti-HBsAg antibodies of serum were detected weekly by ELISA. It was found that the detectable antibodies of transcutaneous immunized mouse were elicited after four weeks, and reached a maximum at the sixth week. Even 1μg plasmid DNA in liposomes through immune skin can elicit the highest ELISA antibody titer (> 1:512) in test group, and corresponding percentage of positive response is up to 71% at sixth week, but higher amounts of plasmid DNA (50μg DNA per mice) on immune skin cannot induce higher antibody levels. The result showed that DNA-liposome complexes glued on bare skin appear to be a novel method for the administration of DNA vaccines.

Keywords: genetic vaccine; liposome; transcutaneous immunization

1. Introduction

Genetic immunization is a simple way to elicit immune response and it is an area in which great progress has been made in this decade [1-3]. Genetic immunization responses vary extremely with different routes of inoculation [4]. The immunology response by the gene gun route on skin is 100 times higher than that of an intramuscular (i.m.) route; only 0.3 μg plasmid DNA can induce antigen specific antibodies and cytotoxic T lymphocytes [5]. It is assumed that the efficient immunization of DNA vaccine needs two major factors: one is the efficient expression of antigen, the other is the process that antigen inducing both antibody against the encoded protein antigen and cytotoxic T cell. The skin and mucosal tissue are the anatomical entry sites where most of exogenous pathogen infect. Several types of skin-associated cell take part in the process of antigen inducing immune response [6-7]; the Langerhans cells of skin carry the antigen from the skin to the draining lymphoid; the dendritic cells and the macrophage of the dermis can also take up antigen and initiate immune response. Therefore, delivery DNA into dermal cells and expression of antigen in these sites would be expected to be an efficient route for gene immunization that mimics a physiologic response to infection [5].

To penetrate the barrier of the epithelium and cause plasmid DNA to reach the skin-associated lymphoid tissue or other viable skin cell to express antigen, several methods have been developed. Gene gun with gold microparticles has been used to physically deliver genes into the cytoplasm of skin cells [1,8]. Intradermal injection of gene immunization could trigger a humoral and cellular immune response [5]. Adenovirus vectors applied onto bare skin can elicit an immune response against the protein encoded by the vector [9]. Our laboratory has found that plasmid DNA can induce higher immunology response by epidermal scratch inoculation than intramuscular and intraparenteral (i.p.) injection routes in mice [10].

Plasmid DNA encapsulated in liposome was used as a DNA delivery system to induce immune responses by intranasal, intramuscular and intraperitoneal immunization [11-13]. In these experiments we found that applying DNA-liposome complexes on bare skin with transdermal delivery enhancer can induce humoral immunization response. And even 1 μg plasmid DNA in liposome can induce HBsAg-specific antibody. The frequency of positive response by skin inoculation is up to 71%. Skin inoculation of DNA-liposome complexes could provide a new potent, painless, and economical rout of DNA immunization.

2. Material and methods

2.1 Plasmid

The mammalian expression vector pGFP,-HBsAg in which HBsAg is inserted into vector pGFP (Clonetech, U.S.A) between GFP and SV40 poly A was constructed by our lab. The structure of pGFP-HBSAg is shown in Figure 1.

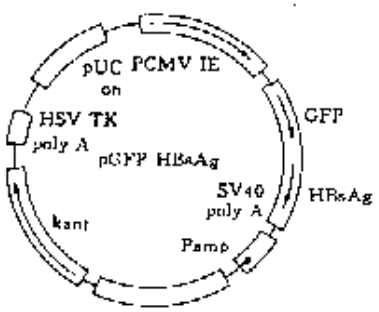

Figure 1. The structure of pGFP-HBsAg plasmid.

2.2 Preparation of DNA-liposome complexes

Phosphatidyl choline-cholesterol-stearylamine (7:2:1) were dissolved in chloroform, and mixed with plasmid DNA and ether. DMSO was added as an enhancer of transdermal delivery system. The organic solvent was removed by rotary evaporators under vacuum until a homogenous aqueous phase appeared. The plasmid DNA liposome was collected for further experiments.

2.3 Inoculation on mice

Mice (BALA/C; 5 weeks old, 7 mice in each experimental group) were anesthetized and hair of an ear was removed with a depilatory (Daen, Spain). DNA-liposome complexes were glued onto the pre-treated area of ear, which was scrubbed with 70% ethanol until the vessels were swollen. The mice were immunized three times at weekly intervals (0, 2, 3). As control, the same amount of DNA-liposome complexes were inoculated by i.p route.

2.4 ELISA for assaying anti-HBsAg antibody levels

Serum was collected by retroorbital bleeding and stored at -70℃. A standard ELISA was performed to determine the HBsAg specific antibodies using purified HBsAg as the capture antigen. The serum samples were diluted in 2-fold increments. The end-point titer was calculated as the dilution of serum producing the same OD_{490} as the same dilution of pre-immune serum. The high positive immunization response was defined as the titer of serum higher than 256.

3. Results

The immunization responses of skin inoculation and i.p route are compared in Figure 2. Even 1μg pDNA on skin immunization can elicit the same level antibody titer as that of i.p route (both reach the highest titer of 512), although i.p immunization can induce antibody response more quickly. The

percent of high positive response by skin inoculation is up to 71% (Table 1), so the skin inoculation can induce efficient immunization response.

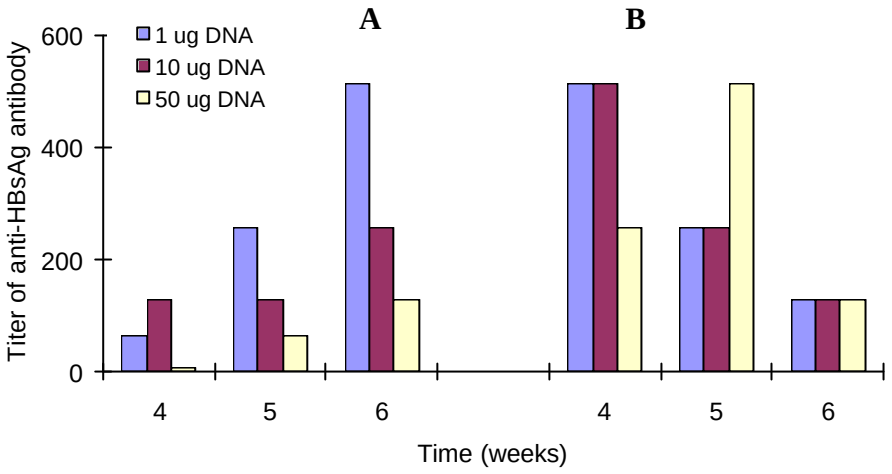

Figure 2. The titer of anti-HBsAg antibody by skin (A) and by i.p inoculation (B).

Table 1. The ratio and the percent of high positive immune responses by skin and i.p inoculation.

	Skin inoculation			i.p inoculation		
Weeks	4	5	6	4	5	6
1 μg DNA	1/7(14%)	3/7(43%)	5/7(71%)	4/6(67%)	4/6(67%)	4/6(67%)
10 μg DNA	3/7(43%)	1/7(14%)	5/7(71%)	4/7(57%)	3/7(43%)	1/7(14%)
50 μg DNA	0/6(0%)	0/6(%)	3/6(50%)	5/7(71%)	7/7(100%)	6/7(86%)

High dose plasmid DNA on skin immunization can't induce high antibody responses as shown in Table 2, and lower dose of DNA-liposome complexes can elicit higher anti-HBsAg antibody response and more frequency of high positive responses. The antibody titer of 1μg DNA is 512 and frequency of high positive response is 71% (5/7) at six weeks, which is higher than those of 50μg. It indicated that skin inoculation maybe is a low dose route of immunization. But whether 1μg is the minimal amount of plasmid DNA necessary for skin inoculation need to determine.

The time of immunity responses of skin inoculation is relatively late. On average, the immunization response by skin inoculation is later 1~2 weeks to reach its highest level of humoral antibody than that of i.p route.

Table 2. The comparison of immune effect of DNA-liposome complexes by skin and i.p inoculation.

	Skin inoculation			i.p inoculation		
	1 μg	10 μg	50 μg	1 μg	10 μg	50 μg
The time of best immune response	6 weeks	6 weeks	6 weeks	4 weeks	4 weeks	5 weeks
The highest titer of antibody	512	256	256	512	512	512
The percent of high positive immune response	71%	71%	50%	66%	57%	100%

4. Discussion

4.1 Penetration of DNA-liposome complexes through skin

Since the DNA-liposome complexes glued on bare skin can invite immunity responses, it is presumed that the DNA-liposome could penetration skin and express antigen under skin, although the antigen expression in skin cell can't be detected by west blotting. The mechanism of penetration of DNA-liposome is unknown. .It is expected that DNA-liposome could penetrate into body via hair follicles, sweat duct or channels in the skin [13-14]. In this DNA-liposome system, the cationic charge on stearylamine could facilitate DNA-liposome adsorbing on skin. And DMSO, as enhancer of transdermal delivery, may contribute to penetration the membrane of skin cell.

Our DNA immunization on skin does not requires needle injection, but the DNA can penetrate the cornified epithelium and reaches the skin-associated-lymphoid tissues or other viable cell. It is hypothesized that the abundance of Langerhans cell or other cells in the skin may uptake the DNA-liposome, express antigen and facilitate the initiation of an immune response, and the dense antigen-presenting macrophage and dendritic cells under the glued skin site may strongly enhance immune responses, particularly at very low antigen concentrations [7, 15, 16]. This may be the reason why low doses of pDNA can elicit high immunization responses. But high doses of pDNA on skin immunization can’t induce high antibody response. It is assumed that higher doses of pDNA may express more antigen protein which may induce immunity tolerance [17].

The response of cytotoxic T cells (CTL) would be tested further. The intradermal gene immunization of free plasmid DNA encoding the influenza nucleoprotein can induce anti-nucleoprotein special antibody and CTL [5]. It is possible that skin inoculation of DNA-liposome complexes also induce antigen-special CTL.

One of our striking results of our DNA vaccine administration is that skin immunization is a efficient method of inoculation. Even 1 μg plasmid DNA can induce antibody response but whether it is the minimum dose of DNA that could stimulate immune response needs to be determined, and more potent immunity responses by DNA-liposome complex on skin might be improved by construction of skin-special expression DNA vaccine vector and developing more efficient transdermal delivery system with potent adjuvant, such as Azone.

DNA-liposome inoculation does not require conventional needle injection and mimics pathogen infections, it is a simple, low dose and safe route of DNA immunization, and may also provide the information about immunity of skin and skin-associated-lymphoid tissue against infections.

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