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DNA Vaccination Using pcDNA3.1 eGFP in Experimental Study and its Effect on Pregnancy and Embryogenesis

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Abstract

DNA vaccination that is for non-human animals has been successful over the past years is a method of protecting animals against infections by injecting them with genetically modified DNA to trigger direct production of antigens by the cells, resulting in an advanced immune protective response. There is remarkable progress and hope towards realizing the DNA vaccines for humans especially again frequent and dangerous disesases although the current statistics do not show any success in developing a functional genetically engineered DNA vaccine for the human race. There are many doubtness about the use of DNA vaccines especially in a special condition as in pregnancy. The aim of this paper is to discuss the effect of DNA vaccines in pregnancy and its effect on embryogenesis.

Keywords: DNA, Vaccine, Pregnancy, Equilibrium.

Introduction

DNA vaccination is a method of protecting human or animals against infections by injecting them with genetically modified DNA to trigger direct production of antigens by the cells, resulting in an advanced immune protective response. The development of DNA vaccines for non-human animals has been successful over the past years. There is remarkable progress towards realizing the DNA vaccines for humans although the current statistics do not show any success in developing a functional genetically engineered DNA vaccine for the human race. There is hope of managing to eradicate dangerous and frequent diseases through the development of suitable DNA vaccines. These conditions range from viral, bacterial, parasitic and several types of tumors [1].

Only one DNA vaccine for humans has approval for utilization in the enhancement of the species up to date. They have a variety of potential advantages and conveniences that the type of vaccines has over the conventional vaccines. They have the ability to eradicate a broad range of immune responses. Their vaccines have a vast of effects on the development of the embryo when the animal is in pregnancy. They affect various aspects of the components of the genetic set up of the animal under development [2].

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Producing DNA Vaccine using pcDNA3.1 eGFP Technic

Gene delivery has the ability which includes vaccination all through to the correction of genes which have mutated. There also exist a variety of different methods that can be used to express genes which are exogenous. Most of the viral vectors have the potential to either integrate a genetic payload into the genetic make-up or portray a gene in an episomal way. However, these viral vectors mostly offer code for the viral proteins which can cause diver stating response by the immune system and may also lead to mortality. Also, there will be an incorporation of those viruses that are in a family of viruses known as a retroviral family in the genome in a random fashion and thus cause mutations in themselves. Another option for gene delivery is known as Naked DNA delivery. These gene delivery methods especially those with explicit expression will last up to about 19 months in vivo in a lot of tissue types which include the muscle tissues will readily in cooperate naked plasmid Deoxyribonucleic acid efficiently and without any complications. Plasmid deoxyribonucleic acid has more advantages as compared to viral vector systems making the delivery paths of the gene to be in equilibrium [3].

To effectively study the functionality of certain processes of physiologic nature, Gene delivery using naked DNA is more appropriate to come up with evidenced conclusion. It will assist the population to shed more light and enhance understanding which can motivate the coming up with a proper management and control of ischemia. It is a disease condition that is associated with the reduction in the supply of blood brought about by the blocking of the blood vessels in a process of occlusion often dangerous for the functioning of tissues. The decrease in the provision of blood could be caused by a disease called atherosclerosis or the accumulation of cholesterol and other fats making arteries lose elasticity. Atherosclerosis, when found to be in the heart, is referred to as coronary artery, and then the subsequent ischemia often leads to heart attack. On the other hand, when atherosclerosis affects the brain it is scientifically known as cerebrovascular disease preceding ischemia often leading to stroke. In other body parts, it's collectively termed as peripheral artery disease and is associated with a lot or severe pain in the muscles evidenced mostly during and immediately after the physical exercise when there is the limited provision of energy by metabolism. To treat ischemia, the most efficient way is the involvement in the physical exercise to burn out accumulated metabolic products like fats in the body [4].

Other methods of treatment may involve complicated medical procedures that include stenting and the bypassing of blocked blood vessels to enhance the continuous blood flow. Since this is a disease of the old age, it is challenging to perform the physical exercise with ease since the intense pain takes the time to subside and make one able to engage in any physical activity. However, the application of stenting and bypass procedures more often than not develops other health defects like the restenosis and the vessel used in the bypass system irrespective of whether it is autologous or artificial are unable to react to external body stimuli. They are not efficient and requires the invention of more alternatives for more efficient therapy or case management.

The body has a natural way and ability to realign damaged arteries and clogged veins in a process scientifically known as arteriogenesis. The flow of blood is promoted to the ischemic regions of the body with studies refuting the existence of such scenarios. Clinical trials, for instance, have taken hypothesis to the test and found out that increasing lateral remodelling is most probable to increase downstream blood supply which doesn't exist in real world situations. The use of experimental animal, the mouse, led to the observation that the remodelling process can prevent the vasodilation of blood arteries and veins which make individuals with ischemia have problems. In this model, PAD is triggered by either recession of the femoral artery or ligation of the same artery. It is then followed by the stimulation of the gracious muscle and profound femoris, side by side vesicles which are undergoing arteriogenesis is evaluated for swelling [3].

With this animal model sometimes it will be hard to assess and analyse the individual process of the process of arteriogenesis which includes the activation of the endothelial cell, proliferation of the smooth muscles and leucocyte extravasation. For this to succeed it is vital to activate firstly without mixing up and make a comprehensive analysis of the vasodilation. It will enable careful application of analytical skills to determine the process that produces the reduced arterial ability to swell. To achive this, utilization of naked deoxyribonucleic acid delivery is vital in driving expression on the mitogens of smooth muscles in an unlilitigated artery.

pcDNA-eGFP production

One of established method to produce pcDNA-EGFP is involved the use of backbone pcDNA3 (A1) and inserted pIRES2-eGFP (A2) as portrayed by application of Sander Lab basically at UCSD. Backbone plasmid contains ubiquitously expressed promoter CMV, on the other hand, the insert plasmid contains the fluorescent reporter probe eGFP. The analysis of plasmids' sequences must be done through an established gold standart. It can be done through the use of LaserGene with the alternative primers that were chosen from bioinformatic properly designed analysis to polymerase chain reaction clone with the restriction sites in the sequence ATCGGGATCCATGGTGAGCAAGGGCGAGGAGCT-3', eGFP-EcoRI: 5'-ATCGGAA TTCTTACTTGTACAGCTCGTCCA-3') were acquired through technology that uses deoxyribonucleic acid technology.

One shot MACH1 competent *E. coli* (Invitrogen) were used to enhance the pcDNA3 and pIRES2-eGFP plasmids through the transformation. *E. coli* were very common found in Indonesia. When the experiment was viewed overnight, there was growing of transformed cell cultures when ampicillin was added to 100 ug/ml for the backbone alone and 50 ug/ml of kanamycin used for the insert plasmids. The performance of mini-preps (Invitrogen) to retrieve the deoxyribonucleic acid from the competent cells. It was followed by a performance of PCR to monitor and track restrictions sites which are of necessity triggering eGFP sequence which was cut by the use of the restriction enzymes *EcoRI* (fermentas) and *BamHI* to make the overhanging sticky ends free. It led to the conduction of purification of Plasmid purification through the use of pure link plasmid purification kit (Invitrogen). The pcDNA3 backbone was then likewise cut with *EcoRI* and *BamHI*, which was separated through electrophoresis of the gel. These were then cut out of the gel and purification followed. Finally, the bands were illuminated by passing ultraviolet radiation to prevent gene mutations [5].

Last but not least, the new pcDNA3-eGFP plasmid is cut by use of *BamHI* and *EcoRI* restriction digest enzymes. DNA is after that separated through gel electrophoresis and imaged to detect any backbone then insert bands. Also important is the PCR method that helps in determining the insert presence. Note that similar primers applied in PCR to clone insert sequence are used in new engineered plasmid pcDNA3-eGFP for production. To drive the expression of eGFP particularly in prokaryotes, the plasmid of pcDNA3.eGFP is turned into BL-21. During this process, bacteria are propagated as described before. In the log phase of growth, BL-21 cells are administered in 10 ml M IPTG for 3 h. After propagation, cells are centrifuged, suspended in 1x PBS and imaged by use of a fluorescent microscope [5].

Testing expression of the plasmid in cells of mammals, a transfection of pcDNA3-eGFP into 3T3 fibroblasts is conducted by use of Mirus also known as TransIT transfection kit. Fibroblasts are grown to roughly 80% confluence in a six-well plate, and later transfection is done with the pCDNA3-eGFP plasmid of about 2.5 ug. Additionally, pNgn3-IRES2-eGFP is also transfected to get 3T3 cells as a positive control. During this transfection, cells are cultured in Invitrogen (Opti-MEM) which is supplemented with 3% of FBS in the absence of antibiotics so that it can prevent cellular toxicity. The cells are then analysed at 24 and 48 h post transfection for the presence of eGFP using fluorescence microscopy.

pDRIVE-eGFP production

Achieving mouse muscle specific expression requires that plasmids of pDRIVE-Myoglobin and pDRIVE-Desmin be purchased from Invitrogen. pDNA is then propagated through overnight culture and after that purified using the mini-prep kit used earlier. Notably, about 10 ug of each plasmid is digested with the restriction endonucleases BamHI and Fermentas. It is to remove the LacZ gene. Digest DNA backbones and LacZ gene are after that separated by use of gel electrophoresis. Illumination of the bands associated backbones is then done using long wave, UV light. It is then cut out manually from the gel with a razor blade. The DNA gel found is then purified by use of the kit for purification. PCR cloning is then conducted on the eGFP insert to add 5' NcoI and 3' EcoRI flanking sequences on the insert (NcoI-eGFP: 5'-ATCGCCATGGTGAGCAA GGGCGAGGAGCT-3', eGFP-EcoRI: 5'-ATCGGAATTCTTACTTGTACAGCTCGTCCA-3').

The new eGFP insert was after that digested by use of BamHI and NcoI endonucleases and purified through PCR purification so that its sticky ends could be revealed. Litigation of the two pDRIVE backbones and eGFP insert are then done. After litigation, pDNA was transformed into chemically competent cells (MACH1) after that plated on LA agar plate with zeocin antibiotic (Mezo, 2012) [5]. Colonies were picked and propagated in 3 ml LB broth plus zeocin overnight. pDNA was mini-prepped and stored a temperature of -20^{o}C.

In the determination of the presence of the insert into the backbone, two diagnostic tests were performed. The pcDNA was first digested with the endonucleases BamHI and NcoI for 1 h at 37^{o}C. Gel electrophoresis was performed to distinguish the backbone and insert. In the determination, if the eGFP was inserted in the correct orientation, PCR using the forward Sh ble and reverse NcoI primers was performed (Shable: 5-GGACTGAGGATAAGAATTGAG-3'). Sh le primer was designed off of a non-coding sequence of DNA upstream of the promoter region of the pDRIVE plasmid.

DNA vaccination in embryogenesis

The first dimension of the embryo development that the process of DNA vaccination alters is the kinetics of the antibody response. The reaction of the humoral after the immunization will be much longer than the answer when the recumbent protein enters the body. The process of DNA vaccination, therefore, develops an extended response time of the embryo against the effects of the hepatitis B virus. On the other hand, it maintains the lifelong protective response towards the influenza haemagglutinin. The experimentation of this feature took place in the mice on the administration of the vaccine [1].

DNA immunization migrates the antibody secretions cells in the embryo under development to the spleen and the bone marrow. It elongates the term of antibody production. They converge at this point after one year. DNA vaccination raises the immune response much more slowly than natural recombination would do. It takes a longer time to reach the peak of production and at the same time increasing the rate of antibody production in the body. The slow response is due to the level of antigens overt the previous weeks of administration [6].

It supports both primary and secondary phases of antibody production. When the DNA vaccine exhibiting HBV protein is injected into adult bodies with hepatitis the vaccine result to specified interferon gamma cell production producing an alteration in the development of the embryo if the adult is pregnant. It, therefore, will lead to the production of specific gamma interferon. It also develops T-cells for the inner envelope antigens. The immune of the host could not attain fit state to monitor the HBV injection [7].

Administration of DNA, a productive adult, would affect the humoral or antibody of the developing embryo. Several factors and conditions influence the responses that antibodies display due to the presence of introduces DNA antibodies. These

factors are the type of antigen that is encoded, the location of the gene that is being expressed in the DNA structure of the embryo under formation, the number of the genes that are expressed. The frequency with which the administration of the vaccine takes place, the dose of the jab, and finally the method and site of delivery of the element also affect the effects of the antibodies. The locations of the antigen expression can be either intracellular or secreted [8].

The feature expresses cytotoxic T-cell response. The vaccines can induce cytotoxic T lymphocytes in the embryo development without affecting the genetic makeup of the fetus being formed in the productive adult. This property eliminates the inheritance risk that may arise. The process permits raising the CTL epitopes against the immuno-dominant and the immuno-recessive epitopes. This method mimics a natural infection [9].

The feature may act as a good way to control the epitopes, therefore, presenting the possibility of providing immunity to the formulated embryo. These cells recognize small, simple peptides and complex molecules in the structure of the developing embryo. These peptides are derivatives of the endogenous cytosolic proteins. They are degraded and delivered to the endoplasmic reticulum in the basic MHC class module. Targeting the gene production thus enhances the CTL response [1].

Developing oocytes gather in their ooplasm RNAs, proteins, organelles, among different segments that are essential amid right on time improvement when the fetus is transcriptionally idle. In this connection, one can expect that oocytes that do not express their qualities may not create to term. This theory is upheld by reports demonstrating that quality expression in oocytes is variable and corresponds well with formative capability. Proof that declaration of particular conditions decide oocyte destiny after preparation was given who discovered 29 qualities differentially communicated in oocytes with high versus low developmental limit. Among these, there were qualities included in translation regulation, RNA handling and protein combination, and corruption. Also, other articulation of conditions involved in DNA copy and repair and additionally cell cycle control recommended a contribution of these qualities with oocyte fitness [10].

Notwithstanding RNA and protein particles, the measure of mtDNA gathered in developing oocytes likewise appears to assume a meaningful part in the improvement. This conviction depends on the vast measure of mtDNA present in adult oocyte and the obvious nonappearance of mtDNA replication amid ahead of schedule advancement. Considered that mtDNA duplicate number relates well with vitality request in somatic cells, oocytes with fewer mtDNA copies may be less capable. In backing of this thought, mtDNA duplicate number differs from a couple of thousands to over a million duplicates among oocytes, and reports are demonstrating a relationship of duplicate number and formative skill, For example, said that barren oocytes.

People have fewer mtDNA copies [1] also, found that mouse oocytes with fewer than 50,000 mtDNA duplicates cannot create to term, bringing about formative capture after embryonic implantation.

In spite of the fact that these outcomes apparently exhibit an impact of mtDNA duplicate number on oocyte capability in a few animal varieties), this result stays to be demonstrated in different species, for example, steers. For example, in a recent report found no relationship between mtDNA duplicate number and developmental ability. The creators assessed mtDNA content from cytoplasmic biopsies expelled from oocytes before parthenogenetic enactment and in vitro society. Accordingly, no distinction of mtDNA duplicate number was found among oocytes that created to the blastocyst stage, oocytes that blocked improvement after cleavage, and oocytes that did not sever. Moreover, even oocytes that contained to a high degree little duplicate numbers (e.g. under 90% of normal mtDNA) could create to the blastocyst stage, giving proof that raised mtDNA duplicate numbers are not required for oocyte developmental capability in dairy cattle) [11].

The above results diverge from prior discoveries, in a taking after test the same creators tried to affirm their outcomes utilizing an alternate experimental methodology in light of evacuation of 64 percentages of oocyte mitochondria by

micromanipulation. In concurrence with their past results, low mtDNA duplicate numbers had no impact on advancement. The creators likewise found that drained extending to develop lives renewed the mtDNA content the replication period, which this way was upheld by expanded articulation of qualities controlling mtDNA. Subsequently, this work further affirms their previous findings were giving confirmation that cow-like incipient organisms are fit for directing mtDNA duplicate number with no impact on mtDNA oocyte content on improvement. As for the relationship between mtDNA duplicate number and oocyte capability reported by others), the creators contended (that species-particular contrasts might have represented the inconsistent results. The mtDNA content has been found not to reproduce until after the blastocyst stage in a few animal categories incorporating mice while this has not been affirmed. We as of late discovered that in the vitro Society of mouse developing lives results in expanded levels of mtDNA in blastocysts (unpublished information), proposing that *in vitro* society might affect replication of mtDNA).

The adequacy of quality weapon organization of non-covering EGFR DNA immunization was the best among three sorts of EFEGFR DNA antibodies and appeared to relate to CD8+ T cells. Along these lines, CD8+ T cells were exhausted with monoclonal counteracting agent 2.43 to figure out if CD8+ lymphocytes were required for the restorative viability. We performed CD8+ T cell exhaustion at week-by-week interims amid the whole test and the convention. Depletion of CD8+ lymphocytes ultimately determines overall survival of mice administrated with non-covering DNA immunization using g, g. Hence, these outcomes suggested that CD8+ T cell assumed a significant part in the interceding restorative adequacy of quality weapon organization of non-covering EGFR DNA antibody [12].

Spleen and lymph hubs were disconnected from vaccinated mice to inspect the particular immunologic cell reaction to Sec-N'- EGFR DNA antibody utilizing diverse organization strategies. The lymphocytes were recolored for the surface CD4, CD8 marker, and intracellular IFN-γ after recombinant human EGFR antigen incitement. Non-covering Sec-N'- EGFR organization by quality firearm generated most practical EGFR-particular CD8+ T cell cells as confirm by their creation of intracellular IFN-γ in the lymph node. Interestingly, splenic lymphocytes segregated from an intramuscular infusion of Sec-N'- EGFR mice bunch had higher useful EGFR-particular CD4+ and CD8+ T cells when contrasted, and g.g DNA and i.m covered gold particles immunized mice groups, individually. What's more, they likewise evaluated cytotoxic T lymphocytes (CTLs) movement in mice inoculated with Sec-N'- EGFR DNA immunization by three distinct techniques. The cytotoxic T lymphocytes (CTLs) effector capacity in spleen gave off an impression of being in the request i.m mice bunch g.g-DNA covered gold particles and g.g-non including DNA mice groups control band (delineated in an individual mouse and as gathering means. In contrast, the percent of particular cytotoxic T lymphocytes lysis in inguinal lymph hub of inoculated mice demonstrated that just non-covering DNA administrated through quality firearm is adequate to actuate CTL effector capacity. Consequently, taken together, the quantity of utilitarian CD4+, CD8+ T cell, and level of CTL action in inguinal lymph hub and spleen were differentially influenced by the courses of organization and detailing of the DNA vaccine.

DNA vaccination is a field that is gaining much prominence in the study of medicine and genetic engineering. It gives hope for a permanent solution to some of the chronic illnesses that affect the human race. Their significant effects on the development are evident. It offers a subunit vaccination that has no risk for infection. It, therefore, is suitable for the development of permanent cure that will not give side effects to its users. The model present antigen presentation by both MHC class II or I [13]. The vaccination can polarize T-cells to help towards realizing type 1 and 2. The DNA immune system focused only on the antigen, which are major participants in disease elimination process. The production of the jab is possible for a limited period. It triggers the synthesis of peptides and purification of recombinant proteins to curb the toxic adjuvant. It provides long-term protection against potential pathogens and development of special features [1].

However, there are weaknesses in this technique. First, it bases only on the protein immunogens thus it may not be useful for the antigen like bacterial polysaccharides. It presents the risk of altering the growth of cells. It is also possible to introduce antibodies that may react with the DNA. The DNA vaccination technology is a promising technology to provide the best solution to current chronic illness and enhancement of the human species. It is important to consider the safety of the process before undertaking.

Sequences are used in new engineered plasmid pcDNA3-eGFP for production. To drive the expression of eGFP particularly in prokaryotes, the plasmid of pcDNA3.eGFP is turned into BL-21. During this process, bacteria are propagated as described before. In the log phase of growth, BL-21 cells are administered in 10 ml M IPTG for 3 hours. After propagation, cells are centrifuged, suspended in 1x PBS and imaged by use of a fluorescent microscope [5]. Testing expression of the plasmid in cells of mammals, a transfection of pcDNA3-eGFP into 3T3 fibroblasts is conducted by use of Mirus also known as TransIT transfection kit. Fibroblasts are grown to roughly 80% confluence in a six-well plate, and later transfection is done with the pCDNA3-eGFP plasmid of about 2.5 ug. Additionally, pNgn3-IRES2-eGFP is also transfected to get 3T3 cells as a positive control. During this transfection, cells are cultured in Invitrogen (Opti-MEM) which is supplemented with 3% of FBS in the absence of antibiotics so that it can prevent cellular toxicity. The cells are then analysed at 24 and 48 h post transfection for the presence of eGFP using fluorescence microscopy.

Conclusion

Polynucleotide vaccines are much preferable as compared to licensed formats which may include stabilization of its temperature, cost affectivity and the potential to encode multiple antigens. Licensed veterinary polynucleotide vaccines are available for cancer in dogs. West Nile virus in the horse, hematopoietic necrosis which is infectious in salmon and reproductive syndrome found in porcine. These are situations where vaccines of the DNA have demonstrated maximum immunogenicity in studying human population.

The study assesses the spread of DNA 3.1 pc EGFP in neonates born to mother mice injected with pcDNA3.1 EGFP. The research method is a technique of modelling with Balb c mice. This research was an experimental laboratory study. Parent mice were divided into two groups, the first group was given a dose of 600 mg/50 µl and the second group was given a dose of 6000 mg/50 µl. Each group is given one-time booster dose equal to that received previously.

The development of the fetus during pregnancy is a complex and rarely examined issues related to ethics. There is evidence that the transfer of IgG, which is identical to the circulation of the mother and the child reported by James E. Crowe Jr., concluded that maternal antibodies can cross the placenta barrier. But not yet reported on any organ neonates IgG expressed from the application of DNA 3.1 pc EGFP. The purpose of this study was to assess the spread of DNA 3.1 pc EGFP in infants born to mother mice injected with pcDNA3.1 EGFP [14].

There are some recommendation for having a good expression of eGFP protein to make observation easier. In all cloning experiment, the vector was dissected with the appropriate restriction enzyme and subjected to 1% agarose gel. Linear vector was removed from the gel through the use of Fermentas DNA extraction from agarose gel kit. Vectors with compatible ends in the chain were dephosphorylated. It enhances the visibility of the DNA strands after being extracted from the gross gel.

Green Fluorescent Protein contains about two hundred and thirty-eight amino acids weighing approximately 27000 atomic mass unit with less than four amino acids able to produce light that is typically seen as glowing forming the necessary or the primary structure. The secondary structure which is a helix strand linked by the hydrogen bonds joining the chains. The tertiary structure is the eleven sheets of barrels closed by the helices at the top. At the center of this structure is composed of a

structure known as the chromophore which contains the disfigured amino acids which are the ones responsible for the color variability always seen with jellyfish and can be cloned to get a lot of copies that can be transfixed into new species. In real life, it can also be used to design machines adaptable to naval expeditions [5].

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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