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Aerosol vaccine of Influenza-A Critical Review

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Abstract

Viruses are the most important mortal pathogens. The influenza virus is accountable for mortality and chronic illness in the world. Today we study the methods of vaccine administration i.e. aerosol method and its evaluation for the improvement of the changes in this method before given to humans and their immunological activity. The aerosol method is taken because it provides the advantages of direct administration of the antigens into bronchi of the lungs thereby producing the antibodies directly for the strains of influenza introduced since its presently nonreplicable viruses. Since vaccination can be done at age of 6 months to 65 years, the aerosol vaccination can be given for the age group of 2 -65 years, it can be self-medicated so that dependence is not required as such of the injectables, and it is directed for localized action and there by systemic action. The study also helps in understanding and better the development of vaccines which are useful for the mankind in future, there by chances of eradicating the deadly diseases, this review helps in understanding of the vaccine developments and also in formation of antigen antibodies, comparative studies between the aerosol vaccines, liquid dosage form and solid dosage form help in better understanding of the design of the vaccinations which is useful for all age groups and also useful for the better recovery from diseases and also the effective means for eradication of the viral infections.

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INTRODUCTION

Vaccines is a biological preparation made from an inactive or killed form of virus which causes disease, in order to provide active acquired immunity against same virus.

Influenza is the highest infection causing virus in the world which mainly affects the upper and lower respiratory track Figure 1. Influenza is also the most contagious disease which spread as flu, and results in serious respiratory illness that may cause death. Constant changes are seen in the structural form of the virus to attain the resistance against the immunity that body has already developed. (To *et al.*, 2016; Santos *et al.*, 2017).

As a result, various government agencies, public health organizations, including the World Health Organization (WHO), have recommended that yearly influenza vaccination be routinely offered, particularly to people at risk of complications of influenza and those individuals who live with or care for high-risk individuals, including,

the elderly (UK recommendation is those aged 65 or above)

people with chronic lung diseases (asthma, COPD, etc.)

people with chronic heart diseases (congenital heart

disease, chronic heart failure, ischaemic heart disease)

people with chronic liver diseases (including cirrhosis)

people with chronic kidney diseases (such as the nephrotic syndrome)

people whose immunity is suppressed (those with HIV or who are receiving drugs to suppress the immune system such as chemotherapy and long-term steroids) and their household contacts

people who live together in large numbers in an environment where influenza can spread rapidly, such as prisons, nursing homes, schools, and dormitories.

healthcare workers (both to prevent sickness and to prevent spread to patients)

pregnant women. However, a 2009 review concluded that there was insufficient evidence to recommend routine use of trivalent influenza vaccine during the first trimester of pregnancy. Influenza vaccination during flu season is part of recommendations for influenza vaccination of pregnant women in the United States (To *et al.*, 2016).

Symptoms for Presence of Viruses

It's generally self-treatable since the symptoms are common cold, fever, chills, cough and running nose.

Patients may also experience Headache, Fatigue, Pain in muscle areas, Dehydration, Loss of appetite, Shortness of breath, Sore throat, Congestion and Swollen Lymph nodes.

The cationic liposomes are produced due to their unique advantages in the process of the immune response. They contain the purified proteins which are favourable for both live and inactive vaccines. By (Table 1) giving the vaccines through the aerosol method the antigens are directly administered into the bronchi of lungs and the production of the antibodies against the given antigens is faster and direct since the respiratory tract mucosal barrier is the strongest route that favours the antibody and cell-mediated immunity development through the layer of mucosal tissues Figure 2, (Santos *et al.*, 2017).

Injection Versus Nasal Spray

Flu vaccines are available either as,

1. a trivalent or quadrivalent intramuscular injection (IIV3 or IIV4, that is, TIV or QIV), which contains the inactivated form of the virus
2. a nasal spray of live attenuated influenza vaccine (LAIV, Q/LAIV), which contains the live but attenuated (weakened) form of the virus.
3. TIV or QIV induce protection after injection (typically intramuscular, though subcutaneous and intradermal routes which can also be protective) based on an immune response to the antigens present on the inactivated virus, while cold-adapted LAIV works by establishing infection in the nasal passages.

Comparative Studies Between Powder and Liquid Dosage

Comparison between the powder dosage forms and the liquid dosage.

Reaching higher deposition efficiency is a feature of the powder dose than the liquid dosage.

For powder aerosol there is no need for the additional or extra driving force.

It's easy for the patients to use than the liquid dosage form.

In previously pre-clinical also the powder inhalation was proven better than liquid aerosol.

The immune response was considered better than the liquid formulations which are delivered intramuscularly or by pulmonary route.

The flow rate of powders can be monitored than the liquid aerosol formulations.

Liquid feed should be sent through the atomization where the process is exposed to higher temperatures, high stress, and dehydration stress.

The flow rates cannot be monitored for the liquid aerosols, hence the dose rate can be also be expected to be vary.

Hence it is recommended that the use of the powder aerosol than the liquid aerosol.

Nasal route over oral route

Nasal route of administration is considered to be the fastest route of approach compared to another route.

The nasal route is opted since it's the fastest and dose can be directly reached to the lungs or respiratory system.

Whereas in the oral route of administration the onset action time is more than considered.

Through oral route the chances of side effects for the other body parts are more (Sham *et al.*, 2004; Saluja *et al.*, 2010).

Formulation Liposomes

According to atal. Slütter B, Bal SM, Ding Z, Jiskoot W, Bouwstra JA the formulation is done as follows,

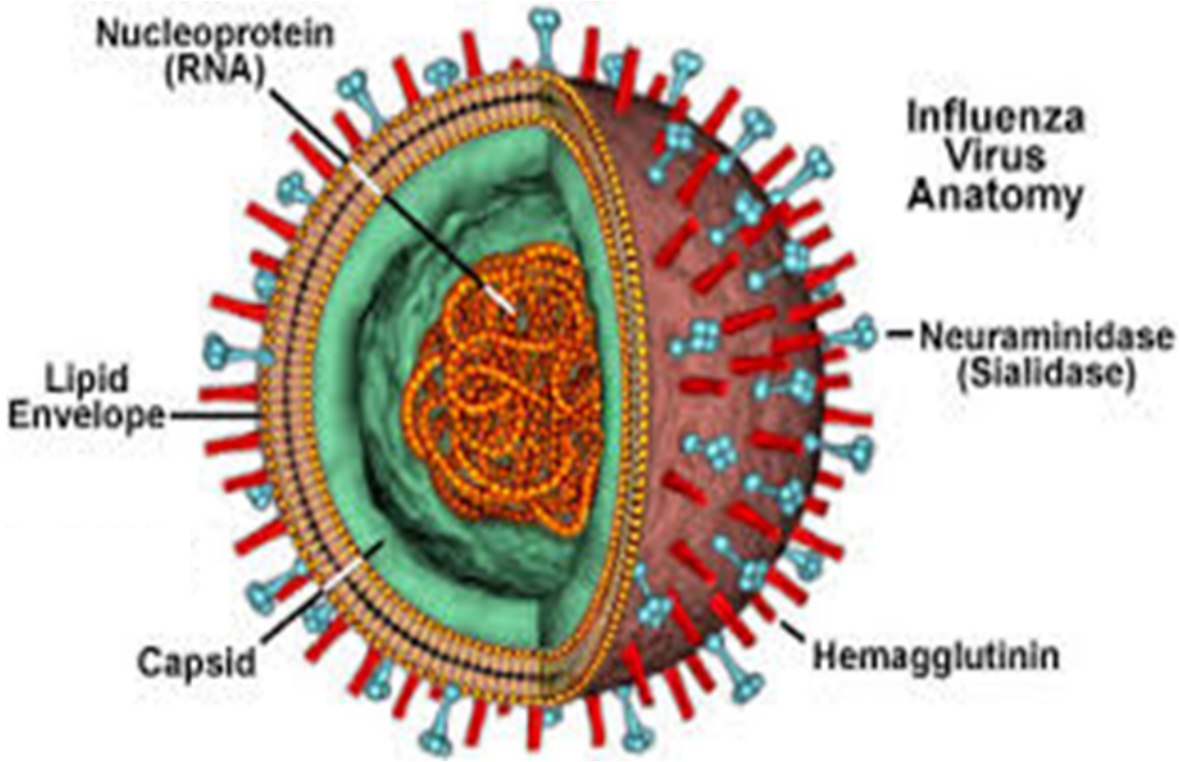

Figure 1: Structure of Influenza Virus

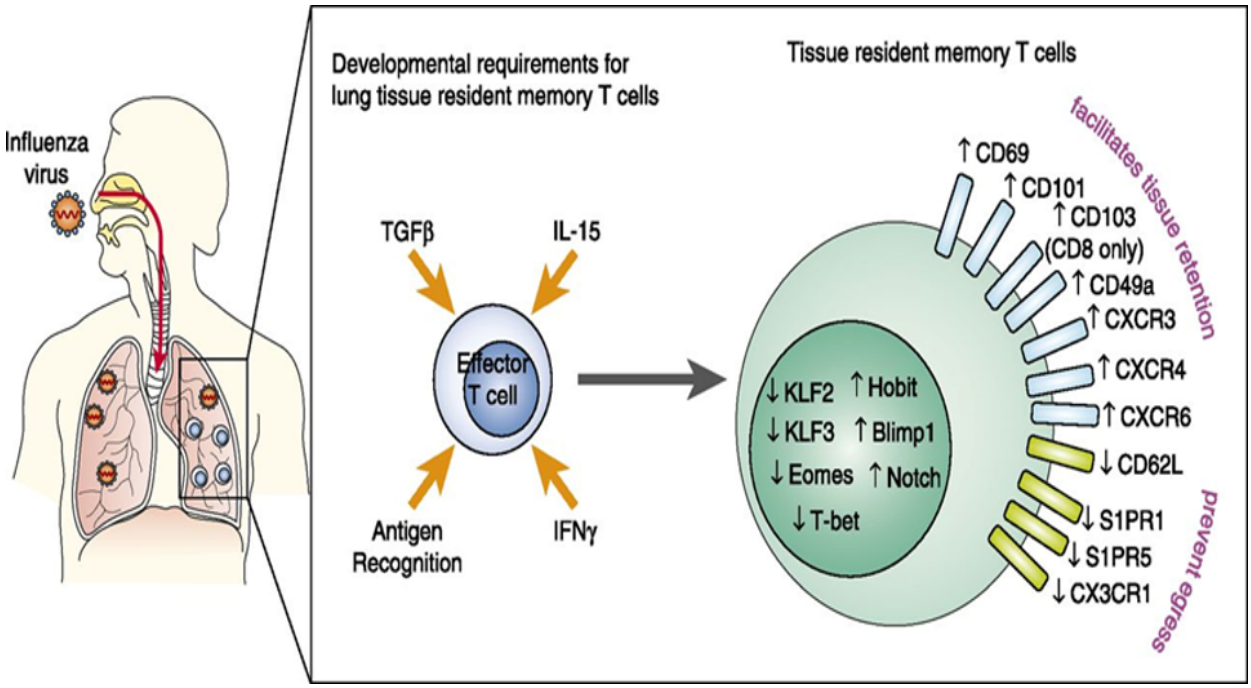

Figure 2: Starting of Infection Process in Humans

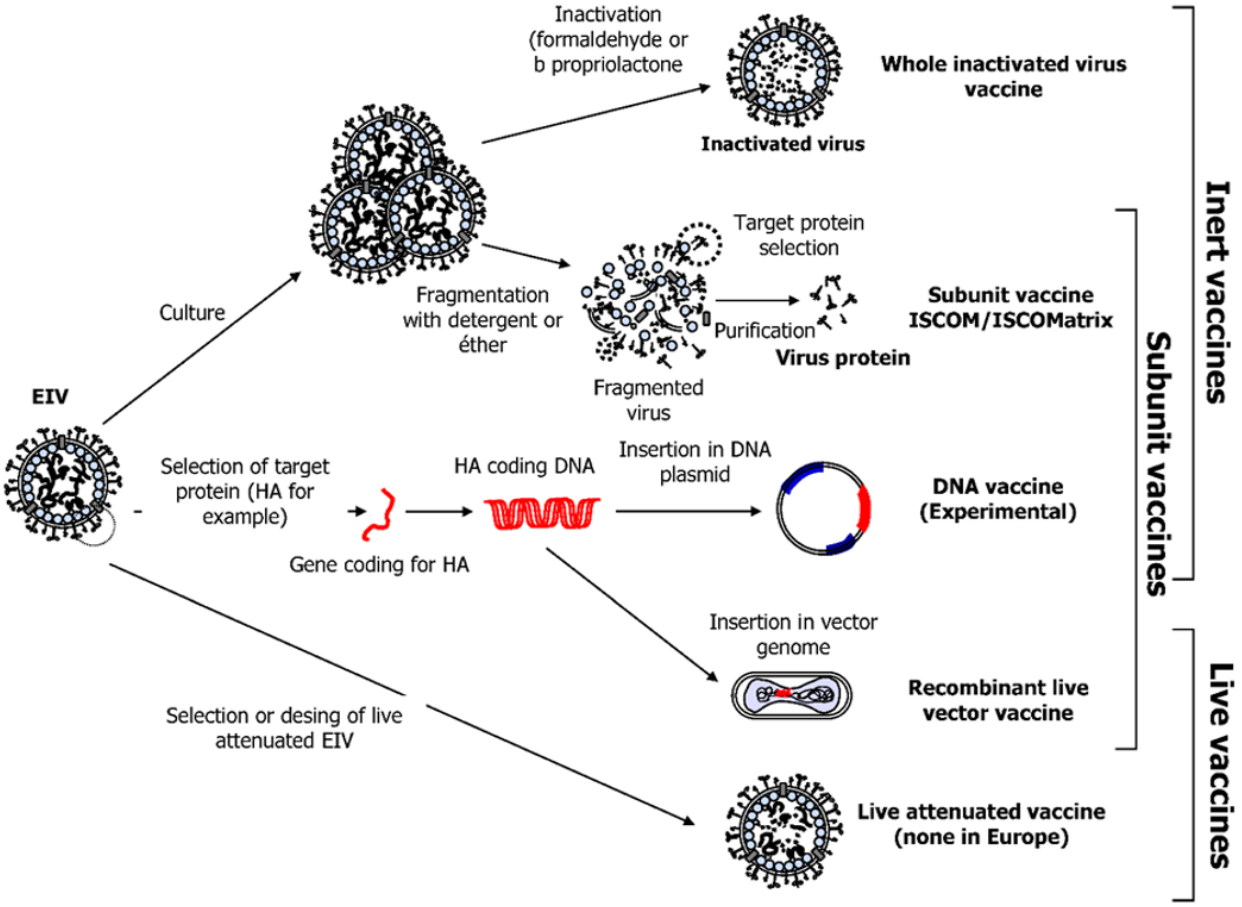

Figure 3: The coding of the DNA of the influenza viruses for the formation of the vaccines

Table 1: Types of Mucosal Vaccines available in Markets

Type of Vaccines	Trade Names for vaccines (Developer)	Route of Administration
Influenza type A and Influenza type B viruses	FluMist (Med Immune)	Intranasal route
H1N1 influenza vírus (swine flu)	NASOVAC (Serum Institute of India)	Intranasal route
Rotavirus	RotaTeq (Merck); Rotarix (Glaxo-Smithkline)	Oral route
Poliovirus	Many	Oral route
Salmonella typhi	Vivotif (Crucell); Ty21A	Oral route
Vibrio cholera	Orochol (Crucell)	Oral route
Cholera	Dukoral (Crucell); Shanchol (Shantha Biotechnics)	Oral route

Table 2: Central Composite Design with independent input variables

S.NO	Hydration volume	Hydration time	Drug: lecithin Ratio	Sonication time(mins)
1	105	30	0.1:3.5	21
2	120	45	0.1:2	14
3	90	45	0.1:2	28
4	90	45	0.1:6	28
5	105	15	0.1:3.5	21
6	90	30	0.1:3.5	21
7	105	30	0.1:6	21
8	90	15	0.1:6	14
9	120	15	0.1:6	28
10	120	45	0.1:6	14
11	105	30	0.1:2	21
12	105	30	0.1:3.5	28
13	120	15	0.1:2	28
14	105	45	0.1:6	21
15	105	30	0.1:3.5	14
16	120	30	0.1:3.5	21
17	90	15	0.1:2	14

Table 3: Vaccine Formulation by Administration route

	OVA (μg)	CpG (μg)	Liposomes (μg)	Total volume (μ1)
Transcutaneous	100	100	5000	70
Nasal	20	20	1000	10
Intradermal	2.0	2.0	100	30
Intranodal	0.20	0.20	10	10

By using the fluorocarbon in water the microparticle shell is prepared using the spray drying process. In this method, the emulsion was made, stabilized by using the saturated lipid as material (Lipoid, Germany) (derivatives of phosphatidylcholine derivatives, DPPC and DSPC; EPC3 previously described (Dellamary *et al.*, 2000; Glück *et al.*, 1999).

During spray drying process, solutions of stephydroethyl starch and antigens are added which are used as hydrophilic agents and lactose and calcium chloride which are used as the other nonactive excipients. The particle size was supposed to be in 1 and 5 and density is about less than 0.2g/cm3 Figure 3.

A similar process is used for the formulation of the Fluzone, which contains the protein antigen and tyloxapol present in 0.5% and 1% respectively.

Characterization

Scanning electron microscopy and densitometry techniques are used for the microparticles description. The process used is as follows,

The sterile buffer PBS is taken and ten SDM were suspended which contained the antigen, which is for the description and they are incubated at 37°C under slow shaking conditions. The samples are formed which have the ranges of the time and centrifuged and the supernatants are stored at -70c. The PBS solution containing the 0.1% SDS and the liquid pallets are resuspended in it and the old shaking at room temperature for a time of 30 mins and the recentrifuged samples are removed from the solution and accordingly, the protein concentration was determined using the assay process and using the bioassay method the TcH specific and HA110-120 peptides are used and cultured with the M12 B Lymph cells used for the antigen percent present.

Preparation Liposomes

Atal. Slütter B, Bal SM, Ding Z, Jiskoot W, Bouwstra JA proposed this method where we can get to know the different methods of preparation of liposomes this

method is as follows,

The film hydration method is used for the preparation of the liposomes (Bangham *et al.*, 1989) which is then concluded with extrusion (Bal *et al.*, 2011). By using the PC, DOTAP, and DOPE film was formed by using the rotary evaporator the chloroform which was added is evaporated and thereby washing with nitrogen. Ratios used is (9:1:1 molar ratio).

By using phosphate buffer pH 7.4 (7.7 mM Na2HPO4 and 2.3 mM NaH2PO4) 10 mM, containing OVA1.5 mg/ml the film was rehydrated. After the lipid formation 5%(w/v), the rehydration process takes place by adding CpG (final concentration of 1.5 mg/ml) and freeze-dried the dispersions were followed by 10 mM phosphate buffer pH 7.4 used in rehydration. extrusion was done for 4 times through two different pore size filters i.e. 400nm and 200nm for the formation of the liposomes in equal size in solution.

Characterization

Using a Zetasizer® Nano ZS (Malvern Instruments, UK), the size of the liposomes can be determined by dynamic light scattering (DLS) and the zeta potential can be measured by laser Doppler velocimetry (Amidi *et al.*, 2006).

Evaluation of novel aerosol formulations

Evaluation test for liposomes

Size reduction

It is evaluated by Sonicator, generally probe sonicator is used for the estimation. Based on the delivery of the powder amplitude, it is set for about 40%, at room temperature and time intervals 14,21,28 mins (Table 2).

Particle size: Its measured by the zeta sizer nano instrument

Determination of Zeta Potential

Instrument used is dynamic light scattering used for the zeta potential estimation (Malvern Instruments Inc., Malvern, UK).It is estimated in room temperature and refractive index used is 1.33. the liposomal solution is added in tubes without air bubbles for their measurement.

Determination of entrapment efficiency

The entrapment of the drug in the liposomes is estimated by using centrifugation technique, the pellets are centrifuged for 4000 rpm for 18 mins there by the drug is released into centrifugation tube and the process is repeated again at 2000 rpm for 38 mins and temparatue is to be 4°C. The drug is mixed with methanol and water and it is estimated by HPLC instrument (Table 2).

Percentage Entrapment Efficiency

$$= \left(\frac{Wc}{Wt}\right) \times 100$$

Determination of Drug loading

The liposomal solution is dissolved in the methanol and water solution there by the solution is used for determination of the drug present in them at various levels its estimated by HPLC instrumentation pipes used are 0.22 micrometers.

Vaccination

8 week old female BALB/c mice (Charles River, Maastricht, The Netherlands) received OVA or OVA- and CpG-containing formulations via transcutaneous, nasal, intradermal or intranodal administration (Table 3).

Animal Studies

The animal studies are performed to evaluate the activity of the formulation prepared and tested and to know if any further changes are requied, which when done, they can be comfortably used for human administration.

According to these studies, the female rate species {BALB} and Sprague Dawley rats are taken for testing from(Indianapolis, IN) Harlan Sprague Dawley, were conserved in the precise microorganism-free, Alliance Pharmaceutical Corp which is qualified of AAALAC. "Principles of Laboratory Animal Care" and are observed in the experiments studied they're and were approved by the Institutional Animal Care and Use Committee (Dellamary *et al.*, 2000).

Advantages of using mice for animal studies

1. They are low cost
2. They are easily available
3. Small in size and easy to handle
4. They can be reared easily
5. They are well suited for the animal studies
6. There is the availability of the transgenic mice and can be easily mutated
7. Well suited for alternative routes of administration.

Antigens

This testing method is important for the vaccines which are to be tested because they are the ones which produce the antibodies for the given antigen strain. The method is as follows,

In this testing method on tolerant (MDBK), the WSN strain of influenza virus are grown on the Madin–Darby bovine kidney carcinoma cells. The A/WSN/32 H1N1 viruses was recuperated and supernatant by sucrose-gradient centrifugation in sterile phosphate buffered saline (PBS), they are resuspended and ultraviolet (UV) short-wave light used for the inactivation which is done twice standard titration on tolerant Madin–Darby canine kidney (MDCK) carcinoma cells can confirm the Inactivation. The amount of viral antigen was measured in a bioassay using M12 B lymphoma cells as antigen-presenting cell and a T cell hybridoma specific for HA 110–120 peptide (Bot *et al.*, 1996) a sterile preparation is made for intramuscular use from the influenza viruses for the influenza vaccines which are administered into the chick embryo, (strains:A/New Caledonia/20/99(H1N1) ,A/Panama/2007/99(H3N2) and B/Victoria/504/2000(ZonalPurified,Subvirion) Fluzone® (AventisPasteur,Swiftwater), PA for the process of inactivation formaldehyde is used on the virus-containing fluids. By the process of the linear sucrose density gradient solution, the influenza viruses are purified after extraction. By using polyethylene glycol Piso-octyl phenyl the virus was chemically disrupted producing a "split-antigen".

In isotonic sodium chloride solution and sodium phosphate-buffered the split-antigen was added and purified by chemical means, by the manufacturer, at a concentration of 45_g hemagglutinin (HA)/0.5 ml dose of Fluzone® was suspended in saline solution.

This method of testing the viruses antigen testing for influenza virus is taken from the review of intranasal vaccination.

According to this method, (H1N1: A/Brisbane/59/07; A/New Caledonia/20/99 and H3N2: A/Brisbane/10/07) and MNA and with NIBSC reference HAI assays were performed delNS1-H5N1 (A/Vietnam/1203/04-like) vaccine candidate viruses (Romanova *et al.*, 2009).

Immunization

According to this method, the immunization can be calculated as follows: this method is taken from the novel aerosol formulation and design. In rats for intra-tracheal insufflation by dry powders, delivered directly into trachea insufflator which contains the 1 mg of powder and given into lungs using 2ml of air, so it reached the pulmonary tree directly. (Penna-Century Inc., Philadelphia, PA).

The antigens are suspended in the sterile saline and given in the lungs using the same pattern. In mice, the parenteral administration carried out subcutaneously, at specified time intervals from the veins of the rats the blood is taken from tail veins with bronchoalveolar fluid harvested by large with heparinized phosphate buffer saline up on animal sacrifice. There are other processes for the immunological studies which are described in another review point of where they are performed as follows.

ELISA (Enzyme-linked immunosorbent assay)

This method is taken from Intranasal vaccination neutralizing mucosal IgA antibodies in humans which induce hetero-subtypic.

Total mucosal IgA and IgG ELISA, specific mucosal IgA and serum IgG as well as using microtiter plates are coated with, anti-human IgA orIgG (Sigma) and IgA detection antibodies (Sigma) or HRP-conjugated anti-human IgG, recombinant hemagglutinin (Sino Biological), were performed as mentioned above (Bot *et al.*, 1996).

Precise ELISA test results were stated in random units (AU/ml)depending on the reference standard with that of the calibration curve (pooled serum or nasal wash) by the 4 parameters logistic fit results were normalized to total IgA of Specific mucosal IgA 1_g (Gen5 v2.1, BioTek) (Morokutti *et al.*, 2014).

HAI (Hemagglutination inhibition assay)

A standard protocol from WHO which it analyzed was applied, 0.8% of human erythrocytes and adding 4 HAU/25_l of virus antigen to serially diluted serum for 2 times (Romanova *et al.*, 2009).

The last serum dilution of the HAI titer is the reciprocal that inhibits hemagglutination. At a 60○ angle plate were analyzed and (Morokutti *et al.*, 2014).

MNA (Microneutralisation assay)

The WHO has set a protocol for the MNA which was adapted (Romanova *et al.*, 2009) for determination of this method.

The samples were serially diluted for 2 times, then virus antigen 100 TCID50/5 _l was added and incubated for 120 ± 30 min.

The assay of the cells s carried out (2 × 104cells/well) i.e.Vero or MDCK were then added and the sample is incubated overnight (20 ± 2 h) before they use acetone for stabilizing. The detection of anti-influenza for A viral infection combination with (Chemicon) in polyclonal goat anti-mouse IgG conjugated withHRP (KPL), nucleoprotein-specific monoclonal mouse antibody and stained with TMB (KPL). Stopped plates (4 M H2SO4)are photo metrically measured (BioTek) at 450 nm and for the reciprocal of the dilution that neutralizes 50%of viruses antigen, the MNA titers are used (Morokutti *et al.*, 2014).

Determination of Serum Secretory IgG1, IgG2a, IgA IgG, and IgG

This method is used for determination of the immunological studies which are conducted for the determination of the immunoglobulins.

The coated Microtiter plates (Nunc, Roskilde, Denmark) placed overnight 4°C pH of 9.4 100Mm carbonate buffer with 100ng of OVA per cell.

Wells was to be blocked with 1% BSA in phosphate buffer solution for a time period 1 h at a temperature of 37 °C.

Thereby OVA-specific antibodies were detected using HRP conjugated goat anti-mouse IgG, IgG1, IgG2a or IgA from the serial dilutions that were taken place for 1.5 h after. The determination of Enzyme activity is done by incubating with TMB/H2O2.

In acetate buffer pH 5.5 at 100 mM for 15 min at room temperature. The reaction is stopped with H2SO42 M and at 450 nm with an EL808 microplate reader absorbance can be checked (Bio-Tek Instruments, Bad Friedrichshall, Germany) (Slütter *et al.*, 2011).

Antigen-Specific DCs in Lymph Node

This is used for the determination of specified antigen in the lymph node which is useful in the treatment of the patients and the method is as follows,

The vaccination given for the mice with the preparation mentioned as per (Table 3), but OVA was substituted with OVAAF647 and CpG with CpGFITC.

The mice are sacrificed for the testing and their lymph nodes are taken, This is done after 4 or 24 hrs of time from vaccination given (for nasal the cervical lymph nodes intradermal and intranodal administration the inguinal and for transcutaneous) and the suspension having single cells are obtained. Cells found were washed with FACS buffer and stained with anti-CD11c-PE-Cy7 to allow detection of DCs.

Using flow cytometry (FACSCantoII, Becton Dickinson), the amount of OVAAF647+ and CpGFITC + DCs was determined (Slütter *et al.*, 2011).

Measurement of T cell response

T cells have greatest significance, the cell mediating immunity hence their action on administration is an important aspect; hence the standard test is performed, which is as follows,

On day 40 after giving a vaccination, the rats which are immunized are taken and their lymph nodes are hygienically separated and used for testing. The cells are isolated and they are stored in the solution of 5 × 106 ml-1for time period of 3 days with antigens or cell culture solution. The amount of the cytokines are measured by (Biosource International, Camarillo, CA).

ELISA test method

In various experimental processes, from immunized mice, pulmonary interstitial APC was isolated by method collagenase digestion, (Sigma) gradient centrifugation and positive selection with (Miltenyi Biotech, Germany) anti-MHC class II magnetic beads.

The Flow cytometric analysis was carried out using PE-tagged anti-CD11c, anti-CD11b and the instrument used is a FACS Caliber.

The quantitation of activated TcH was carried and Incubation of HA-specific T cell hybridoma with APC cells and out as described previously (BD PharMingen, San Diego, CA) (Santos *et al.*, 2017).

In some cases, M12 B lymphoma cells (H-2d) pulsed ex vivo with antigens or microparticle extracts obtained are SDS-free, were used as APC (Smith *et al.*, 2003).

CONCLUSIONS

Form the above-discussed review methods, the aerosol method of giving vaccination is preferable and they help in the fast development of the antibodies against the given antigen strain of the influenza viruses and it gives the most effective method of the drug delivery into the respiratory tract and thereby having an excellent bio-availability in the lymphoid tissues. It also helps in the fast recovery of the problems caused.

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