PATENT SPECIFICATION (11) 1 582 763

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(21) Application No. 24913/77 (22) Filed 15 Jun. 1977 (19)

(61) Patent of Addition to No. 1461188 dated 26 Sept. 1974

(31) Convention Application No. 0168041 (32) Filed 18 Jun. 1976 in

(33) Belgium (BE)

(44) Complete Specification Published 14 Jan. 1981

(51) INT. CL.[3] A61K 39/145

(52) Index at Acceptance
A5B 112 132 136 AA

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(54) LIVE INFLUENZA VIRUS VACCINE AND PREPARATION THEREOF

(71) We, SMITH KLINE-RIT formerly known as RECHERCHE ET INDUSTRIE THERAPEUTIQUES RIT of Rue du Tilleul 13, 1320 Genval, Belgium, a Company incorporated under the laws of Belgium, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-

The present invention relates to an attenuated influenza virus vaccine effective on intranasal administration and to the preparation thereof and it represents an improvement in or modification of the invention described in British Patent Specification No. 1461188.

A process for preparing stable influenza virus (H_3N_2) strains completely resistant to serum inhibitors and useful as intranasal vaccines has been described in our patent specification No. 1461188. According to said specification, these strains are obtainable by passaging, in the allantoic cavity of embryonated chicken eggs in the presence of normal serum, a recombinant strain previously obtained from an H_3N_2 influenza virus strain and the A/PR8/34 influenza virus strain. In particular, a virus strain previously obtained by recombining the A/England/42/72 influenza virus strain with the influenza virus strain A/PR8/34 was rendered resistant to serum inhibitors and the resistant strain was used in the preparation of live vaccines.

It is known that, almost every year, the serotype of the type A influenza virus strains spreading in the world is slightly different from the serotype of the previously observed strains.

This antigenic modification causes a specific problem for immunization against type A influenza virus.

In order to obtain the greatest efficacy from a vaccinal antigen, one must be as close as possible to the dominant virus and therefore the influenza vaccines must be adapted at regular intervals to confer the strongest protection against these new strains.

The present invention relates to such improvement. By submitting to three passages in the allantoic cavity of embryonated chicken's eggs and in the presence of normal guinea pig serum, according to the process described in British Patent Specification 1,461,188 an influenza virus strain (H_3N_2) previously obtained by recombing the A/PR8/34 influenza virus with the A/Victoria/3/75 influenza virus, there was obtained an influenza virus strain completely resistant to serum inhibitors, having the A/Victoria/3/75 serotype and valuable for vaccinal use.

The so-obtained strain, named RIT 4050, has been deposited in the Collection of viruses held by the WHO Collaborating Centre for Collection and Evaluation of Data on Comparative Virology at the Institut für Medizinische Mikrobiologie, Infektions- und Seukenmedizin der Ludwig-Maximillians Universität at München (Bundesrepublik Deutschland) with the designation P/76/7.

Thus, the present invention provides an attenuated influenza vaccine effective on nasal administration containing as active component an effective dose of an influenza virus strain (subtype H_3N_2) which is attenuated and resistant to serum inhibitors, wherein the serum inhibitor resistant strain is the P/76/7 influenza virus strain. A dosage unit of the vaccine contains at least $10^7 EID_{50}$ (dose infective in 50% of the inoculated eggs) of virus.

The present invention also provides a process for preparing such an attenuated vaccine, which comprises incubating the P/76/7 serum inhibitors resistant influenza virus strain in the allantoic cavity of embryonated hen's eggs, and incubation being for a period of time

sufficient to permit growth of a large amount of said virus and harvesting the resulting virus material which is preferably supplemented with a stabilizer -peptone is an example of stabilizer- and freeze-dried.

For the administration, the vaccine, which is preferably kept in freeze-dried form, is extemporaneously reconstituted either by addition of water or by addition of any other pharmaceutical diluent or composition known to the art for the preparation of nasal preparations such as drops or spray and inoculated in the nasopharynx. If desired, a second dose is inoculated one to two weeks after the first administration.

The type A influenza virus vaccine of this invention can be used in combination with any other live influenza virus vaccine -e.g. a type B influenza virus vaccine- administrable by nasal route.

The following Examples illustrate the present invention but they should not be construed as limiting its scope.

EXAMPLE 1

Preparation of the recombinant virus strain

An influenza virus (H_3N_2) strain (designated RIT 4057 in our collection) sensitive to serum inhibitors obtained by recombining the A/PR8/34 strain with the A/Victoria/3/75 strain and cloned by one end-dilution passage is used as starting viral material. Different dilutions (i.e. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) of said starting viral material in normal saline with a titre of $10^{7.5}$ EID_{50}/ml are mixed with a same volume of sterile normal guinea pig serum (in normal saline) previously adjusted at the 1/2 dilution and then maintained for 10 minutes in a water bath at 75°C, homogenized and centrifuged at 1000 g for 30 minutes. The supernantant is used for the further step which consists of incubating the virus serum mixtures at room temperature (20°C) for one hour before inoculating 0.2 ml aliquots of said mixture into the allantoïc cavity of embryonated hen's eggs.

The eggs are then further incubated for a period of time varying between 24 and 96 hours at 37°C.

At the end of this incubation period, the allantoïc fluid is harvested and tested for the presence of influenza virus by the hemagglutination method. The harvested virus produced by the inoculum of the highest virus dilution which shows hemagglutinating activity (i.e. virus dilution 10^{-3}) is used for a second passage performed in the same operative conditions. The harvested virus produced by the inoculum of the highest virus dilution (i.e. 10^{-9}) with guinea pig normal serum and which shows hemagglutinating activity is used for a third passage performed in the same operative conditions.

The harvested virus produced by the inoculum of the highest virus dilution (i.e. 10^{-8}) with guinea pig normal serum and which shows hemagglutinating activity has been designated P/76/7 and characterized as follows.

Inhibitors-resistance

For testing the resistance against the inhibitors present in normal heated animal serum (horse, guinea pig and calf serums previously heated at 75°C for one hour), serial twofold dilutions of the heated serums were mixed with four hemagglutinating units of the parent strain and the modified virus. After incubation for one hour at room temperature chicken red blood cells were added and the serum dilutions giving an inhibition of hemagglutination were recorded. The results are shown in the following Table I.

TABLE I

Serum of	*Serum dilution inhibiting hemagglutination*	
	RIT 4057	*P/76/7*
guinea pig	>4000	<10
horse	>4000	<10

Table I shows that the P/76/7 strain is completely resistant to the serum inhibitors.

Stability of the inhibitors-resistant character

The resistant character of the P/76/7 strain has been confirmed in laboratory animals (ferrets) : the virus isolated 3 and 6 days after inoculation ($10^{7.6}$ EID_{50}) was found resistant to serum inhibitors.

Absence of side effects

A first group of 4 ferrets was inoculated intranasally with $10^{7.6}$ EID_{50} of the P/76/7 strain. The temperature of the animals was taken daily during 6 days p.i. No significant temperature rise was recorded using the criteria determined by the C.W. Potter & al. (Br.J.Exp. Pathol. *53* : 153-67, 1972). A second group of 3 ferrets was inoculated intranasally with $10^{7.6}$ EID_{50} of the RIT 4057 strain. Two animals showed significant temperature rise.

EXAMPLE 2

Preparation of the vaccine

The virus material of the last passage of the process described in Example I is used as inoculum for the production of the seed lot for the manufacturing of the vaccine.

The allantoïc fluids are harvested, pooled, sterility and safety tested, mixed with peptone in order to reach a final concentration of 5 % of peptone. Aliquots of 0.5 ml are distributed into 3 ml glass vials in order to obtain dosage units (i.e. at least 10^7 EID_{50}) of virus. The product is then freeze-dried and the vials tightly stoppered.

For vaccine administration, the contents of one vial are reconstituted by adding 0.5 ml of a diluent such as water or saline or a 5 % sucrose solution and administered as drops in the nostrils.

EXAMPLE 3

Clinical trials

Dosage units ($10^{7.3}$ EID_{50}) of the vaccine obtained following the process described in Example 2 have been reconstituted by adding 0.5 ml of a sterile 5 % sucrose solution and 5 drops per nostril were administered to 15 healthy volunteers (mean age : 22 years).

A second dose was inoculated to 8 of the 15 volunteers 7 days after the first administration.

All the volunteers were examined daily to detect eventual influenza symptoms. Blood samples were collected before, and 2 to 3 weeks after inoculation to determine the number of seroconversions and the geometric mean of the hemagglutination inhibition (HI) titre.

The results of the clinical trials are summarized in the following Tables II and III.

TABLE II

SYMTOMS	*NUMBER (duration in days)*
conjunctival reaction	1 (3)
sore threat	1 (2)
stuffy nose	6 (1)
rhinorrhea	1 (1)

TABLE III : Serology

Number of doses	*Number of seroconversion/* number of vaccinated	geometric mean titre HI (x)
1	12/15	37
2	8/ 8	ND (xx)

(x) titre HI before vaccination ≤ 20
(xx) ND : not determined

As showed in Table II and III, the influenza virus strain P/76/7 is attenuated for humans and induces a high rate of seroconversion.

WHAT WE CLAIM IS:-

1. An attentuated influenza virus vaccine effective on intranasal administration comprising an effective amount of serum inhibitors resistant strain of H_3N_2 influenza virus, wherein the serum inhibitors resistant strain is the P/76/7 virus strain.
2. An attenuated influenza virus vaccine according to claim 1, wherein the effective amount is at least 10^7 EID_{50} of the P/76/7 strain per dosage unit.
3. An attenuated influenza virus vaccine according to either of claims 1 and 2, wherein the vaccine is supplemented with a stabilizer and freeze-dried.
4. A process of preparing an influenza virus vaccine according to any of claims 1 to 3, which comprises incubating in the allantoic cavity of hen's embryonated eggs the influenza virus serum inhibitors resistant strain P/76/7 and harvesting the resulting virus material.
5. A process according to claim 4, substantially as hereinbefore described in Example 2.
6. An attenuated influenza virus vaccine, when prepared by a process according to either of claims 4 and 5.

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Printed for Her Majesty's Stationery Office, by Croydon Printing Company Limited, Croydon, Surrey, 1980.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY,from which copies may be obtained.