PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 38/44, 38/47, G01N 33/569, C12N 9/14, 9/36

A1

(11) International Publication Number: WO 00/50069

(43) International Publication Date: 31 August 2000 (31.08.00)

(21) International Application Number: PCT/US99/04063

(22) International Filing Date: 25 February 1999 (25.02.99)

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US Not furnished (CON)
Filed on 25 February 1999 (25.02.99)

(71) Applicant *(for all designated States except US)*: NEW HORIZONS DIAGNOSTICS, INC. [US/US]; 9110 Red Branch Road, Columbia, MD 21045-2014 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: FISCHETTI, Vincent [US/US]; 448 Joan Court, West Hempstead, NY 11552 (US). LOOMIS, Lawrence [US/US]; 11374 Buckelberry Path, Columbia, MD 21044 (US).

(74) Agents: SANDERCOCK, Colin, G. et al.; Foley & Lardner, *Suite 500, 3000 K Street, N.W., Washington, DC* 20007-5109 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: A MEANS FOR THE PROPHYLACTIC AND THERAPEUTIC TREATMENT OF STREPTOCOCCAL INFECTIONS

(57) Abstract

The present invention relates to compositions containing Group C streptococcal phage associated lysin enzyme for the prophylactic and therapeutic treatment of Streptococcal infections, including the infection commonly known as strep throat. Methods for therapeutically and prophylactically treating such infections also are described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

A MEANS FOR THE PROPHYLACTIC AND THERAPEUTIC TREATMENT OF STREPTOCOCCAL INFECTIONS

PCT

DESCRIPTION

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a composition containing Group C Streptococcal phage associated lysin enzyme and a carrier for delivering the Group C Streptococcal phage associated lysin enzyme to the mouth, throat or nasal passages of a mammal. The composition is particularly useful for the prophylactic and therapeutic treatment of Streptococcal infections, including the infection commonly known as strep throat.

2. Description of the Prior Art

Group A streptococci have been shown to be an important pathogen capable of existing both in a carrier state in an asymptomatic individual and in a symptomatic individual with symptoms of disease ranging from a mild sore throat, tonsillitis, or impetigo. If untreated, these streptococcal infections could lead to glomerulonephritis, rheumatic fever and possibly permanent rheumatic heart disease. With the advent of antimicrobial agents, specifically penicillin derived antibiotics, the causative organism can be readily eliminated following the prescribed regimen of appropriate antibiotic therapy.

The fact that an infected individual (usually children & young adults) can pass group A streptococcal organisms to others, particularly in daycare centers and schools, necessitates the isolation of the known infected individual away from these environments for at least 24 to 72 hours after antimicrobial therapy has been initiated. It has been shown in controlled studies that early detection and appropriate treatment results in a reduction in the overall pattern of cyclic transmission of the troublesome pathogen, as well as a reduction or elimination of the sequelae of group A infections (rheumatic fever or nephritis).

U.S. Patent No. 5,604,109 (Fischetti et al.) teaches the rapid and sensitive detection of Group A streptococcal antigens by a diagnostic assay which utilizes Group C streptococcal phage associated lysin enzyme. Such an assay can assist in rapidly identifying infected individuals, who then can receive conventional antibiotic therapy.

However, the problem of effectively combating streptococcus infections remains a problem. Patients with a streptococcal pharyngitis are highly infectious and are able to transmit the organism to close contacts resulting in an epidemic of strep throat. This is a particular problem in a school population and in the military, where people are in close proximity with one another. Moreover, there is a need for prophylactic methods of preventing streptococcal infections, because prophylactic use of antibiotics generally is not advisable for many reasons.

SUMMARY OF THE INVENTION

The present invention (which incorporates U.S. Patent No. 5,604,109 in its entirety by reference) provides compositions containing Group C Streptococcal phage associated lysin enzyme and a carrier for delivering the Group C Streptococcal phage associated lysin enzyme to the mouth, throat or nasal passage of an animal such as a human. Such compositions can be particularly useful either as a prophylactic treatment for preventing infection following exposure to others who are infected, or as a therapeutic treatment of Streptococcal A throat infections for those who are already infected, thereby alleviating the infection.

The presence of the lysin in an oral or nasal cavity at the time when streptococci are introduced from an infected individual thus results in the killing of at least some of the incoming streptococci, thereby providing a prophylactic effect by preventing infection. This rapid and specific (lethal) activity of the lysin enzyme against an existing streptococcus infection will have a therapeutic effect by reducing the infection and helping to decrease the spread of the strep to other individuals.

In one embodiment of the invention, the lysin enzyme is administered in the form of a candy, chewing gum, lozenge, troche, tablet, a powder, an aerosol, a liquid (e.g., gargle) or a liquid spray. In another embodiment of the invention, the lysin enzyme is administered in the form of a nasal spray.

The method for the treatment of streptococcus A exposure comprises applying an effective dosage of a pharmaceutically acceptable amount of Group C streptococcal phage associated lysin

enzyme to the oral or nasal mucosa of a mammal in need of treatment, permitting the lysin enzyme to remain in contact with the oral mucosa for a period of time necessary for the lysin enzyme to saturate the oral mucosa; and applying additional dosages of such the lysin enzyme in like fashion until treatment is complete. The enzyme may be administered to the mucosal lining of the throat, or of the nose, for the infection, depending on the carrier containing the lysin enzyme.

While this composition is likewise effective against group A strep infections, it may also be used against group C infections. The enzyme is likewise effective against group E streptococci.

DETAILED DESCRIPTION OF THE INVENTION

It has been discovered that the presence of the lysin in an oral cavity at the time when streptococci are introduced from an infected individual results in the killing of the incoming streptococci, thus preventing infection and providing a prophylactic treatment for preventing strep infection. This rapid and specific (lethal) activity of the lysin enzyme against streptococcus also produces a therapeutic effect in infected individuals by reducing the infection and decreasing the spread of the strep to other individuals.

The amidase muralytic (lysin) enzyme produced by the group C streptococcal organism after being infected with a particular bacteriophage (identified as C1) is isolated and harvested as is described in U.S. Patent Application No. 5,604,109. This Group C streptococcal enzyme, (also known as a lysin enzyme) which has

unique specificity for the cell wall of groups A, C, and E Streptococci, may alternatively be isolated and harvested by any other known means.

The composition which may be used for the prophylactic and therapeutic treatment of a strep infection includes the lysin enzyme and a means of application, (such as a carrier system or an oral delivery mode) for the mucosa lining of the oral or nasal cavity.

Prior to, or at the time the enzyme is put in the carrier system or oral delivery mode, it is preferred that the enzyme be in a stabilizing buffer environment for maintaining a pH range between about 4.0 and about 8.0, more preferably between about 5.5 and about 7.5 and most preferably at about 6.1.

The stabilizing buffer should allow for the optimum activity of the lysin enzyme. The buffer may be a reducing agent, such as dithiothreitol. The stabilizing buffer may also be or include a metal chelating agent, such as ethylenediaminetetracetic acid disodium salt, or it may also contain a phosphate or citrate-phosphate buffer.

To prevent spoilage, the stabilizing buffer may further contain a bactericidal or bacteriostatic agent as a preservative, such as a small amount of sodium benzoate.

Means of application include, but are not limited to direct, indirect, carrier and special means or any combination of means. Direct application of the lysin enzyme to the nasal membranes may be by nasal sprays, nasal drops, nasal ointments, nasal washes, nasal injections, nasal packings or through the use of ointments applied to the nasal nares, the bridge of the nose, or the face

or any combination of these and similar methods of application, or other known nasal carriers. Application to the mouth and throat may be made by use of throat lozenges, or through use of mouthwashes, gargles, solutions, sprays, candy, gum, etc. Thus, forms in which the lysin enzyme may be administered include but are not limited to lozenges, troches, candies, injectants, chewing gums, tablets, powders, sprays, liquids, ointments, and aerosols.

The lozenge, tablet, or gum into which the lysin enzyme is added may contain sugar, corn syrup, a variety of dyes, non-sugar sweeteners, flavorings, any binders, or combinations thereof. Similarly, any gum based products may contain acacia, carnauba wax, citric acid, corn starch, food colorings, flavorings, non-sugar sweeteners, gelatin, glucose, glycerin, gum base, shellac, sodium saccharin, sugar, water, white wax, cellulose, other binders, and combinations thereof.

Lozenges may further contain sucrose, corn starch, acacia, gum tragacanth, anethole, linseed, oleoresin, mineral oil, and cellulose, other binders, and combinations thereof. In another embodiment of the invention, sugar substitutes are used in place of dextrose, sucrose, or other sugars.

The enzyme may also be placed in a nasal spray, wherein the nasal spray is the carrier. The nasal spray can be a long acting or timed release spray, and can be manufactured by means well known in the art.

Any of the carriers for the lysin enzyme may be manufactured by conventional means. However, it is preferred that any mouthwash or similar type products not contain alcohol to

prevent denaturing of the enzyme. Similarly, when the lysin enzyme is being placed in a cough drop, gum, candy or lozenge during the manufacturing process, such placement should be made prior to the hardening of the lozenge or candy but after the cough drop or candy has cooled somewhat, to avoid heat denaturation of the enzyme.

The enzyme may be added to these substances in a liquid form or in a lyophilized state, whereupon it will be solubilized when it meets a liquid body. The enzyme may also be, for example, in a micelle or liposome, or some other encapsulated form, or administered as a prodrug or in an extended release form to provide a prolonged storage and/or delivery effect.

The effective dosage rates or amounts of the lysin enzyme to treat the infection will depend in part on whether the lysin will be used therapeutically or prophylactically, the duration of exposure of the recipient to the Streptococci, and/or the nature of the infection, the size and weight of the individual, etc. Determining appropriate dosage rates will be within the skill of the artisan. The duration for use of the composition containing the enzyme also depends on whether the use is for prophylactic purposes, wherein the use may be, for example, daily or weekly, for a defined time period e.g., for a week month or longer, or whether the use will be for therapeutic purposes, wherein a more intensive regimen of the use of the composition likely would be employed for a period of, for example, 2, 3, 4, 5, 6, 7, 10, or 14 days, or longer. Thus, therapeutic treatments could span several days or weeks, likely on a daily basis, and possibly at multiple intervals during the day.

In order to be effective, the enzyme should be present in an amount sufficient to provide an effective number of enzyme units in contact with the mouth, throat or nasal passage. Having too few enzyme units in contact with the mouth, throat or nasal passage, even over a long period of time, will not produce as beneficial an effect as desired. Hence, any dosage form employed should provide for an approximate minimum number of units for the amount of time that the dosage will provide enzyme to the mouth, throat, or nasal passage. The concentration of the active units of enzyme believed to provide for an effective amount or dosage of enzyme may be in the range of from about 100 units to about 100,000 units in the environment of the nasal and oral passages. Within that broader range, dosages of from about 100 units to about 10,000 units are believed to be acceptable. Such units can be contained in smaller volumes of carrier such as liquid or saliva, e.g. 1 ml or less (e.g. in the case of a lozenge) or can be contained in larger dosage volumes such as a gargle of several mls. Generally, therefore, larger volumes of carrier will require a greater total number of units to achieve an effective concentration of active enzyme. Hence, acceptable concentrations can be from about 100 units/ml to about 100,000 units/ml of fluid in the environment of the nasal or oral passages. Within this range, concentrations from about 100 units/ml to about 10,000 units/ml are acceptable.

In practice, therefore, the time exposure to the active enzyme units likely will influence the desired concentration of active enzyme units employed in the dosage per ml. For example, carriers that are considered to provide prolonged release

(certain nasal sprays, lozenges and encapsulated enzyme) could provide a lower concentration of active enzyme units per ml, but over a longer period of time. Conversely, a shorter duration treatment (e.g., a gargle) could provide a higher concentration of active enzyme units per ml. Any dosage form containing sufficient lysin enzyme to provide effective concentrations of active enzyme at the site of infection or to provide a sufficient prophylactic effect are well within the bounds of routine experimentation and therefore, well within the scope of the instant invention.

Compositions of the instant invention further may comprise at least one other additional agent effective for use in either therapeutic or prophylactic treatment of streptococcal infections or in the alleviation of symptoms thereof. The agent may additionally be effective for some additional therapeutic and/or prophylactic effect, e.g. pain alleviation. Some agents can be topically or systemically active, and can include antibiotics or pain alleviation medications including topical antiseptics, e.g., for sore throat. Alternatively, such agents may be given prior to or after treatment with the instant compositions. Dosages of the antibiotics should be effective for therapeutic or prophylactic treatment of streptococcal infections or for treatment of symptoms of such infections.

While this treatment may be used in any animal or mammalian species, the preferred use of this product is for a human.

Each dose of the lysin containing carrier is kept in contact with the oral mucosa as long as necessary in order to provide the intended therapeutic or prophylactic effect.

Administration of the lysin enzyme to the oral mucosa may be by any means such as gargles, mouth rinses, lozenges, troches, chewing gums, candies, powders, and nasal and oral sprays, so long as it is safe and provides the intended prophylactic or therapeutic effect. Skilled artisans may appreciate other possible methods and compositions for delivering the lysin enzyme in accordance with this invention.

EXAMPLE 1

The group C phage lysin enzyme is prepared as follows: Group C streptococcal strain 26RP66 (ATCC #21597) or any other group C streptococcal strain is grown in Todd Hewitt medium at 37 degree(s) C. to an OD of 0.23 at 650 nm in an 18 mm tube. Group C bacteriophage (C1) (ATCC #21597-B1) at a titer of 5X10 sup 6 is added at a ratio of 1 part phage to 4 parts cells. The mixture is allowed to remain at 37 degree(s) C. for 18 min at which time the infected cells are poured over ice cubes to reduce the temperature of the solution to below 15 degree(s) C. The infected cells are then harvested in a refrigerated centrifuge and suspended in 1/300th of the original volume in 0.1M phosphate buffer, pH 6.1 containing 5X10 sup -3 M dithiotreitol and 10 µg of DNAase. The cells will lyse releasing phage and the lysin enzyme. After centrifugation at 100,000X g for 5 hrs to remove most of the cell debris and phage, the enzyme solution is aliquoted and tested for its ability to lyse Group A Streptococci.

The number of units/ml in a lot of enzyme is determined to be the reciprocal of the highest dilution of

enzyme required to reduce the OD650 of a suspension of group A streptococci at an OD of 0.3 to 0.15 in 15 minutes. In a typical preparation of enzyme 4X10 sup 5 to 4X10 sup 6 units are produced in a single 12 liter batch.

Use of the enzyme requires a minimum number of units of lysin enzyme per test depending on the incubation times required. The enzyme is diluted in a stabilizing buffer containing the appropriate conditions for stability, maximum enzymatic activity. The preferred embodiment is to use a lyophilized lysin enzyme. The stabilizing buffer can comprise a reducing reagent, which can be dithiothreitol in a concentration from 0.001M to 1.0M, preferably 0.005M. The stabilizing buffer can comprise a metal chelating reagent, which can be ethylenediaminetetraacetic acid disodium salt in a concentration from 0.00001M to 1.0M, preferably 0.005M. The stabilizing buffer can comprise a citrate-phosphate buffer in a concentration from 0.001M to 1.0M, preferably 0.05M. The stabilizing buffer can have a pH value in the range of from about 4.0 to 8.0, preferably 6.1. The stabilizing buffer can comprise a bactericidal or bacteriostatic reagent as a preservative. Such preservative can be sodium azide in a concentration from 0.001 percent to 0.1 percent, preferably 0.02 percent.

The preparation of phage stocks for lysin production is the same procedure described above for the infection of phage and group C streptococcus in the preparation of the lysin enzyme. However, instead of pouring

the infected cells over ice, the incubation at 37 degree(s) C. is continued for a total of 1 hour to allow lysis and release of the phage and also enzyme in the total volume. In order for the phage to be used for subsequent lysin production the residual enzyme must be inactivated or removed to prevent lysis from without of the group C cells rather than phage infection.

EXAMPLE 2

The enzyme prepared according to example 1 is diluted to a concentration of 100 units/ml in a buffer consisting of 0.05M citrate phosphate buffer pH 6.1 containing 0.1% rabbit immunoglobulin, 0.005M (ethylenedinitrilo) tetraacetic acid disodium salt (EDTA), 0.005M Dithiothreitol, 0.02% sodium azide, 0.01% N-acetylglucosamine. One part colloidal gold sol labelled with Group A Streptococcal Antibody (OD sup 520 1.5) suspended in 0.02M Tris buffer pH 8.2, 1.0% bovine serum albumin, 0.02% sodium azide, 300K units heparin, is added to 3 parts of the enzyme reagent, mixed, filtered through a 0.22 micron filter, and 200 microliters aliquoted per tube and lyophilized. This lyophilized enzyme is stable at elevated temperatures (i.e. 45 degree(s) C.) for short term conditions (i.e. 2 weeks) and long term storage at room temperatures (>1 year).

EXAMPLE 3

Method

1. Start a day culture of group A streptococcal strain S43/192/39R (Streptomycin resistant) (from frozen blood broth); 500 µl in 50 ml of Todd Hewitt (TH) broth containing 1 % yeast extract and 100 µl of Streptomycin/ml.

2. Grow to an OD_{650} of 0.59

3. Centrifuge for 15 minutes at 3000 rpm to sediment bacteria.

4. Resuspend organisms in 1 ml volume of TH w/o antibiotics (3 x 10^5/100 µl as determined by plate count).

5. Add 0.5 of these concentrated cells to).5 ml of pH 6.1 phosphate buffer as a control.

6. Five minutes before administering to the mice, 0.5 ml of the concentrated cell suspension was mixed with 0.5 ml of phage lysin solution pre-diluted to 10,000 units/ml in pH 6.1 phosphate buffer.

Five mice received 60 µl of "control" solution divided equally orally and intranasally.

Five mice received 60 µl of lysin and bacteria mixture divided equally orally and intranasally.

Throat swabs were performed onto 5% sheep blood, proteose peptone agar plates containing 500 µg/ml of streptomycin. Plates were incubated overnight at 37° C.

The following results were obtained:

	7/22 1d	7/23 2d	7/24 3d	7/28 7d
	Colony Forming Units			
LYSIN				
L1	0	0	0	0
L2	0	0	0	0
L3	0	0	0	0
L4	0	1	0	0
L5	0	0	1	0
CONTROL				
C1	26	14	7	0
C2	>400	17	100	83
C3	9	0	15	0
C4	>400	>400	>400	220
C5	2	2	30	0

These results show that the contact between phage lysin and group A streptococci for as little as five minutes prevents the streptococci from colonizing the upper respiratory tract of the mice in this model system.

EXAMPLE 4

To reproduce what might occur when lysin is present in the oral cavity (i.e. through relase by a lozenge) before streptococci enter the environment, animals, bacteria, and lysin were prepared in the same way as described in example 3 (numbers 1-5) except in this experiment, animals were treated as follows:

Lysin: 5 mice received 25 μl of lysin and immediately thereafter they received 50 μl of bacterial suspension.

Control: 5 mice received 25 μl of buffer and immediately thereafter they received 50 μl of bacterial suspension.

24 hrs.

Lysin

L6	-
L7	-
L8	-
L9	-
L10	-

Control

C6	-
C7	+
C8	-
C9	+
C10	+

Result: 0/5 animals containing lysin in their oral cavity when the streptococci were added were colonized, while 3/5 animals that received buffer, then streptococci were colonized. Thus, lysin, if present before group A Streptococci are added, is able to prevent colonization.

EXAMPLE 5

Group A M type 6 streptococci were grown at 37 degrees C overnight in Todd Hewitt media. Organisms were washed once in sterile lysin buffer (50 ml phosphate buffer pH 6.1). The cell pellet was then suspended in 5 ml of the same buffer.

Phage lysin was diluted in lysin buffer containing 5 mM DTT to two times the appropriate units and the mixture was sterile filtered.

1.0 ml of the bacterial suspension was added to 1.0 ml of the appropriate lysin dilution and the mixture was incubated

at 37 degrees C. Samples were removed at timed intervals, diluted appropriately and plated on blood agar plates to determine the bacterial count.

Control samples consisted of 1.0 ml of the bacterial suspension added to 1.0 ml of the lysin buffer alone. An aliquot was removed and diluted in 10-fold dilutions and an aliquot plated on blood agar plates to determine the bacterial count.

Results:

Lysin units	Starting Count	Bacterial counts with lysin: 5 sec	30 sec	60 sec	5 min	10 min	
1000	5×10^6	0	0	0	0	0	(mean 2 exp)
100	8.6×10^6	1530	1196	771	64	6	(mean 4 exp)
10	9.8×10^6	>3000	>3000	>3000	>3000	>3000	(mean 3 exp)

Many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood within the scope of the appended claims the invention may be protected otherwise than as specifically described.

What is claimed is:

1. A composition for use in the therapeutic or prophylactic treatment of a streptococcal infection, comprising:

an effective amount of Group C streptococcal phage associated lysin enzyme; and

a carrier for delivering said lysin enzyme to a mouth, throat, or nasal passage.

2. The composition according to claim 1, wherein said carrier is selected from the group consisting of candy, chewing gum, lozenge, troche, tablet, powder, aerosol, liquid, liquid spray, nasal spray and nasal ointment.

3. The composition according to claim 1, further comprising a buffer that maintains pH of the composition at a range between about 4.0 and about 9.0.

4. The composition according to claim 3, wherein said buffer maintains the pH of the composition at range between about 5.5 and about 7.5.

5. The composition according to claim 3, wherein said buffer comprising a reducing agent.

6. The composition according to claim 5, wherein said reducing agent is dithiothreitol.

7. The composition according to claim 3, wherein said buffer comprises a metal chelating agent.

8. The composition according to claim 7, wherein said metal chelating agent is ethylenediaminetetraacetic disodium salt.

9. The composition according to claim 3, wherein said buffer is a citrate-phosphate buffer.

10. The composition according to claim 1, further comprising a bactericidal or bacteriostatic agent as a preservative.

11. The composition according to claim 1, wherein said lysine enzyme is lyophilized.

12. The composition according to claim 1, wherein said carrier further comprises a sweetener.

13. The composition according to claim 1, wherein the carrier provides a concentration of from about 100 to about 100,000 active enzyme units per milliliter of fluid in the environment of nasal or oral passages.

14. The composition according to claim 13, wherein said concentration is from about 100 to about 10,000 active enzyme units per milliliter of fluid in the environment of the nasal or oral passages.

15. The composition according to claim 1, wherein said composition is used in the therapeutic treatment of streptococcal infections.

16. The composition according to claim 1, wherein said composition is used in the prophylactic treatment of streptococcal infections.

17. The composition according to claim 15, wherein said streptococcal infection is a streptococcal throat infection.

18. The composition according to claim 1, wherein said carrier is a candy.

19. The composition according to claim 1, wherein said carrier is a chewing gum.

20. The composition according to claim 1, wherein said carrier is a lozenge.21. The composition according to claim 1, wherein said carrier is a troche.

21. The composition according to claim 1, wherein said carrier is a troche.

22. The composition according to claim 1, wherein said carrier is a powder.

23. The composition according to claim 1, wherein said carrier is an aerosol.

24. The composition according to claim 1, wherein said carrier is a liquid spray.

25. The composition according to claim 1, wherein said carrier is a nasal spray.

26. The composition according to claim 1, wherein said mammal is a human.

27. The composition according to claim 1, wherein said carrier is suitable for delivering said lysin enzyme to the mouth and throat.

28. The composition according to claim 1, wherein said carrier is suitable for delivering said lysin enzyme to the nasal passage.

29. The composition according to claim 1, further comprising at least one agent effective for the therapeutic or prophylactic treatment of streptococcal infections or alleviation of symptoms thereof.

30. A method of therapeutically or prophylactically treating streptococcal infections comprising administering a composition in accordance any one of claims 1-29 to a mammal in need thereof.

31. The use of Group C streptococcal phage associated lysin in the manufacture of a pharmaceutical composition according to any one of claims 1-29.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/04063

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/44, 38/47; G01N 33/569; C12N 9/14, 9/36

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7, 29, 34, 30, 36, 195, 206, 961, 962, 975; 424/94.1; 94.6, 94.61; 436/518, 524, 531, 533, 536, 808

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	US 5,604,109 A (FISCHETTI et al.) 18 February 1997, see entire document.	1-9,11, 15-17 --- 10, 12-14, 18-31
Y	US 3,852,424 A (GAEUMANN et al.) 03 December 1974, see entire document, particularly, col. 12, line 62 through col. 13, line 7.	1-31

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date		
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search
03 MAY 1999

Date of mailing of the international search report
21 JUN 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
IRENE MARX
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/04063

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7, 29, 34, 30, 36, 195, 206, 961, 962, 975; 424/94.1; 94.6, 94.61; 436/518, 524, 531, 533, 536, 808

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, JPOABS, EPOABS, CAPLUS, BIOSIS, MEDLINE, AGRICOLA
search terms: streptococc?, page associated lysin?; carrier, nose or nasal, throat, mouth, oral; spray, ointment, liquid, aerosol; gum

[19]中华人民共和国国家知识产权局

[51]Int. Cl[7]
A61K 38/44
A61K 38/47 G01N 33/569
C12N 9/14 C12N 9/36

[12]发明专利申请公开说明书

[21]申请号 99816592.1

[43]公开日 2002年6月5日

[11]公开号 CN 1352567A

[22]申请日 1999.2.25 [21]申请号 99816592.1
[86]国际申请 PCT/US99/04063 1999.2.25
[87]国际公布 WO00/50069 英 2000.8.31
[85]进入国家阶段日期 2001.10.25
[71]申请人 新眼界诊断公司
地址 美国马里兰州
[72]发明人 V·菲舍蒂
L·洛米斯

[74]专利代理机构 中国专利代理(香港)有限公司
代理人 关立新 钟守期

权利要求书2页 说明书10页 附图页数0页

[54]发明名称 预防性和治疗性治疗链球菌感染的方法

[57]摘要

本发明涉及含有C组链球菌嗜菌体相关性细胞溶素酶的组合物，所述组合物用于预防性和治疗性治疗链球菌感染包括通常所称的链球 菌咽喉炎。本发明还记载了治疗性或预防性治疗这样的感染的方法。

ISSN1008-4274

权 利 要 求 书

1. 用于预防性或治疗性治疗链球菌感染的组合物，包括:

有效量的C组链球菌嗜菌体相关性细胞溶素酶; 和

用于把所述细胞溶素酶释放于口、喉或鼻腔的载体.

2. 权利要求 1 的组合物，其中所述载体选自: 糖果、口香糖、锭剂、糖锭、片剂、粉剂、气雾剂、液体、液体喷雾剂、鼻喷雾剂和鼻软膏.

3. 权利要求 1 的组合物，其还含有维持所述组合物 pH 为约 4.0-约 9.0 的缓冲物质.

4. 权利要求 3 的组合物，其中所述缓冲物质维持该组合物的 pH 为约 5.5-约 7.5.

5. 权利要求 3 的组合物，其中所述缓冲物质含有还原剂.

6. 权利要求 5 的组合物，其中所述还原剂为二硫苏糖醇.

7. 权利要求 3 的组合物，其中所述缓冲物质含有金属螯合剂.

8. 权利要求 7 的组合物，其中所述金属螯合剂为乙二胺四乙酸二钠盐.

9. 权利要求 3 的组合物，其中所述缓冲物质为柠檬酸盐磷酸盐缓冲剂.

10. 权利要求 1 的组合物，其还含有作为防腐剂的抗菌剂和杀菌剂.

11. 权利要求 1 的组合物，其中所述细胞溶素酶是冷冻干燥的.

12. 权利要求 1 的组合物，其中所述载体还含有甜味剂.

13. 权利要求 1 的组合物，其中在鼻或口腔环境中，所述载体提供浓度约 100-约 100,000 活性酶单位/毫升流体.

14. 权利要求 13 的组合物，其中在鼻或口腔环境中，所述载体提供浓度约 100-约 10,000 活性酶单位/毫升流体.

15. 权利要求 1 的组合物，其中所述组合物用于治疗性治疗链球菌感染.

16. 权利要求 1 的组合物，其中所述组合物用于预防性治疗链球菌感染.

17. 权利要求 15 的组合物，其中所述链球菌感染为链球菌咽喉感染.

18. 权利要求 1 的组合物，其中所述载体为糖果。

19. 权利要求 1 的组合物，其中所述载体为口香糖。

20. 权利要求 1 的组合物，其中所述载体为锭剂。

21. 权利要求 1 的组合物，其中所述载体为糖锭。

22. 权利要求 1 的组合物，其中所述载体为粉剂。

23. 权利要求 1 的组合物，其中所述载体为气雾剂。

24. 权利要求 1 的组合物，其中所述载体为液体喷雾剂。

25. 权利要求 1 的组合物，其中所述载体为鼻喷雾剂。

26. 权利要求 1 的组合物，其中所述哺乳动物为人。

27. 权利要求 1 的组合物，其中所述载体适合把所述细胞溶素酶释放至口和喉。

28. 权利要求 1 的组合物，其中所述载体适合把所述细胞溶素酶释放至鼻腔。

29. 权利要求 1 的组合物，其还含有有效地用于治疗性或预防性治疗链球菌感染或减轻其症状的至少一种试剂。

30. 预防性或治疗性治疗链球菌感染的方法，包括：把权利要求 1-29 任意一项的组合物给药至需要的哺乳动物。

31. C 组链球菌嗜菌体相关性细胞溶素在制备权利要求 1-29 任意一项的药物组合物中的应用。

说　明　书

预防性和治疗性治疗链球菌感染的方法

发明背景

1、发明领域

本发明涉及含有 C 组链球菌噬菌体相关性细胞溶素酶和载体的组合物，其中所述载体用于把该 C 组链球菌噬菌体相关性细胞溶素酶释放至哺乳动物的口、喉或鼻腔．所述组合物特别适用于预防性和治疗性治疗链球菌感染包括通常称之为链球菌咽喉炎的感染．

2、现有技术描述

已知对于无症状个体或者患有轻度喉咙痛、扁桃腺炎或脓疱病的带症状个体来说，A 组链球菌已经显示为能够以载体状态存在的重要病原体．如果不予以治疗，这些链球菌感染能够导致肾小球性肾炎、风湿热和可能永久性的风湿性心脏病．随着抗生素特别是青霉素类抗生素的问世，按照合适的抗生素治疗处方方案，病原生物容易得到消除．

由于被感染的个体（通常为儿童和年轻人）能够把 A 组链球菌传递给其他人，特别是在托儿所和学校，因此必须在抗微生物治疗开始后至少 24-72 小时把已知的被感染个体从这些环境中分离开．在对照研究中表明：早期诊断和合适的治疗能够使令人讨厌的病原体的总循环传染降低，并且能够降低或减少 A 类感染的后遗症（关节热或肾炎）．

美国专利 US 5,604,109（Fischetti 等）教导：通过应用 C 组链球菌噬菌体相关性细胞溶素酶进行诊断分析，能够对 A 组链球菌抗原进行快速和灵敏检测．这种检测能够有助于迅速鉴定被感染个体，然后使被感染个体接受常规抗生素治疗．

然而，还存在一个问题就是是否能有效抵抗链球菌感染．患有链球菌咽炎的患者具有高度传染性，并且能够把病原体传递至密切接触者，从而导致脓毒性咽喉炎流行．这个问题在人群密度较大的人与人密切接触的学校和军队显得尤为突出．而且，需要防止链球菌感染的预防方法，这是因为由于多方面原因，通常的抗生素预防应用是不合适的．

发明概述

本发明（其引用美国专利 US 5,604,109 中的内容作为参考）提供

含有 C 组链球菌噬菌体相关性细胞溶素酶和载体的组合物，所述载体用于把该 C 组链球菌嗜菌体相关性细胞溶素酶释放至动物例如人的口、喉或鼻腔．这样的组合物特别能够用作：预防性治疗以防止在接触已感染个体后被感染，或者对已经被感染的患者进行链球菌 A 喉感染的治疗性治疗，从而减轻感染．

由于口腔或鼻腔中存在细胞溶素，因此当链球菌从被感染个体引入时，至少有一些侵入的链球菌被杀死，因此通过防止感染达到预防效果．通过降低感染以及促进减少链球菌传播至其它个体的方式，细胞溶素酶这种快速和特异性（致死性）抗已有链球菌感染的活性具有治疗效果．

在本发明的一个方案中，细胞溶素酶的给药形式为：糖果、口香糖、锭剂、糖锭、片剂、粉末、气雾剂、液体（例如含漱剂）或液体喷雾剂．在本发明另一个实施方案中，细胞溶素酶以鼻喷雾剂给药．

治疗链球菌 A 感染的方法包括：把有效剂量的可药用量 C 组链球菌嗜菌体相关性细胞溶素酶施用于需要治疗的哺乳动物的口腔或鼻腔，让细胞溶素酶接触口腔粘膜并停留必要的一段时间从而使其饱和口腔粘膜；然后以同样的方式施用另外剂量的这种细胞溶素酶直至治疗完成时为止．根据含有细胞溶素酶的载体的不同，所述酶可以被给药至喉或鼻的粘膜壁（mucosal lining）．

除了能够有效地抵抗 A 组链球菌感染外，所述组合物还能以类似方式用于抵抗 C 组链球菌感染．并且，所述酶还能以类似方式抵抗 E 组链球菌．

发明详述

据发现当链球菌从被感染个体侵入时，细胞溶素在口腔中停留一段时间能够杀死侵入的链球菌，从而预防感染并提供预防性治疗以防止链球菌感染．在被感染的个体中，通过降低感染以及减少链球菌传播至其它个体的方式，细胞溶素酶这种快速和特异性（致死性）抗链球菌感染的活性还产生治疗效果．

被特定的噬菌体（称 C1）感染后，C 组链球菌生物体产生酰胺酶 muralytic（细胞溶素）酶，将其分离并收集，这些方法记载于第 5,604,109 号美国专利中．或者，通过任意其它已知方法，也可以分离和收集这种 C 组链球菌酶（也称为细胞溶素酶），这种酶对 A、C 和 E

组链球菌的细胞壁具有独特的特异性.

用于预防性和治疗性治疗链球菌感染的组合物含有：细胞溶素酶和应用工具（例如载体系统或口服给药模式）以释放于口腔或鼻腔粘膜壁.

当把这种酶加入到载体系统或口腔给药模式中期间或之前，优选把该酶置于稳定的缓冲环境中用于维持 pH 范围在约 4.0-约 8.0、更优选约 5.5 至约 7.5 和最优选约 6.1.

所述稳定的缓冲剂应该使细胞溶素酶显示最佳活性. 该缓冲剂可以是还原剂例如二硫苏糖醇. 所述稳定的缓冲剂还可以含有或可以是金属螯合剂例如乙二胺四乙酸二钠盐，或者其也可含有磷酸盐或柠檬酸盐酸磷酸盐缓冲剂.

为了防止变质，所述稳定的缓冲剂还可含有抗菌剂或抑菌剂作为防腐剂，例如其可含有少量苯甲酸钠.

应用方式包括但不限于直接、间接、载体和特殊方式或者任意方式的组合. 把细胞溶素酶直接应用至鼻粘膜可以通过的方式为鼻喷雾剂、滴鼻剂、鼻软膏、洗鼻剂、鼻注射液、鼻填充物或者通过把软膏直接施用于鼻孔、鼻桥或面部或者上述方式的任意组合，或者通过类似的应用方法或其它已知的鼻载体施用. 施用于口腔或喉部可以通过的方式为应用喉锭剂或通过应用漱口剂、含漱剂、溶液剂、喷雾剂、糖果和胶等. 因此，可以施用细胞溶素的形式包括但不限于锭剂、糖锭、糖果、注射剂、口香糖剂、片剂、粉剂、喷雾剂、液体制剂、软膏和气雾剂.

加有细胞溶素酶的锭剂、片剂或胶可以含有糖、玉米浆、各种染料、非糖甜味剂、矫味剂、任意的粘合剂或这些物质的组合. 类似地，任意胶基产品可以含有阿拉伯胶、巴西棕榈蜡、柠檬酸、玉米淀粉、食品着色剂、矫味剂、非糖甜味剂、明胶、葡萄糖、甘油、胶基、紫胶、糖精钠、糖、水、白蜡、纤维素、其它粘合剂以及上述物质的组合.

锭剂还可以含有糖、玉米淀粉、阿拉伯胶、黄蓍胶、茴香脑、亚麻籽、含油树脂、矿物油和纤维素、其它粘合剂以及这些物质的组合. 在本发明另一实施方案中，应用糖取代物替换葡萄糖、蔗糖或其它糖.

还可以把该酶置于鼻喷雾剂中，其中鼻喷雾剂为载体. 所述鼻喷

雾剂可以是长期作用的喷雾剂或暂时释放的喷雾剂，并且可以通过本领域公知的方式制备.

所有这些用于细胞溶素酶的载体均可以通过常规方式制备，但优选任何漱口剂或类似产品不含有乙醇以防止酶变性. 类似地，在制备过程期间，当把细胞溶素酶置于止咳糖、胶、糖果或锭剂中时，应该在锭剂或糖果硬化之前但在止咳糖或糖果稍微冷却后将细胞溶素酶放置好，这样可以避免酶变性.

可以把所述酶加入到这些液体形式或冻干状态的物质中，因此所述酶遇到液体时会被溶解. 所述酶也可以是例如微胶粒或脂质体形式，或者其它被包封形式，或者以前药形式或缓释形式给药以便提供延长的储存期和/或释放效果.

治疗感染的细胞溶素酶的有效剂量或含量将部分取决于以下因素：该细胞溶素酶是用于预防还是治疗、用药者接触链球菌的持续时间、和/或感染的性质、个体体积和重量等. 合适剂量的确定容易由本领域技术人员确定. 含有该酶的组合物的应用持续时间也取决于以下因素：所述应用是否用于预防目的，用于预防目的时所述应用可以是例如每天或每周，持续确定的时间例如 1 周、1 月或更长；或者所述应用是否用于治疗目的，当用于治疗目的时，可能应用更加强的方案例如应用 2、3、4、5、6、7、10 或 14 天或更长. 因此，治疗性治疗可能横跨数天或数周，可以以天为基础，并可能在一天期间有多个间隔.

为了达到效果，所述酶应该以足够量存在以提供有效数目的酶单位来接触口、喉或鼻腔. 如果接触口、喉或鼻腔的酶单位太少，即使是长时间接触也不会产生需要的有益效果. 因此，所有应用的剂型应该在一段时间内提供大约最少量的数量单位，这样所述剂型将把酶提供至口、喉、或鼻腔. 在鼻腔或口腔环境中，被认为提供有效含量或剂量活性单位酶浓度的范围是约 100 单位至约 100,000 单位. 据信，从约 100 单位至约 10,000 单位的较宽范围是可以接受的. 这样的单位可以存在于小体积载体中例如 1ml 或更小体积的载体例如液体或唾液中（例如锭剂），或者也可以存在于大体积例如若干毫升含漱剂中. 因此，通常载体的体积越大，达到活性酶有效浓度所需的单位总数越大. 因此，在鼻或口腔中，可接受浓度可以是约 100 单位/毫升至约 100,000 单位/毫升流体中. 在此范围内，约 100 单位/毫升至约 10,000 单位/

毫升的浓度是可以接受的.

因此，在实践中，接触活性酶单位的时间可以影响活性酶单位在每毫升剂量中所需要的浓度. 例如，被考虑提供缓释的载体（某些鼻喷雾剂、锭剂和被包封酶）能够提供每毫升较低浓度的活性酶单位，但持续时间较长. 相反，较短的持续治疗（例如含漱剂）能够提供每毫升较高浓度的活性酶单位. 所有在感染部位提供有效浓度活性酶或提供足够预防效果的含有足够细胞溶素酶的任何剂量完全属于常规实验范围之内，因此完全在本发明范围之内.

本发明的组合物还可含有至少一个其它添加剂，所述添加剂能有效地用于治疗性或预防性治疗链球菌感染或者减轻其症状. 另外，这些试剂还可以有效地用于某些其它治疗和/或起预防作用，例如减轻疼痛. 一些试剂可以是局部活性或全身活性，并可含有抗生素或疼痛减轻药物包括例如局部用抗菌剂用于减轻咽喉痛. 或者，可以在应用本发明组合物处理之前或之后给予这样的试剂. 抗生素的剂量应该能有效地用于治疗性或预防性治疗链球菌感染或用于治疗这些感染的症状.

当这些治疗用于任意的动物或哺乳动物时，优选把这些产品应用于人.

让含有各个剂量细胞溶素的载体接触口腔粘膜必要的时间以便提供期望的预防或治疗效果. 把细胞溶素酶给药至口腔粘膜的方式可以为任意方式，例如：含漱剂、口腔清洗剂（mouth rinses）、锭剂、糖锭、口香糖、糖果、粉剂、和鼻或口腔喷雾剂，条件是其安全并其能提供期望的预防或治疗效果. 根据本发明，本领域技术人员可以知道用于给药细胞溶素酶的其它可能方法和组合物.

实施例 1

C 组嗜菌体细胞溶素酶制备如下：在 18mm 试管中，在 37℃下的 Todd Hewitt 培养基中把 C 组链球菌株 26RP66（ATCC#21597）或任意的 C 组链球菌株培养至其在 650nm 处的 OD 为 0.23. 以 1 份嗜菌体比 4 份细胞的比例把 C 组噬菌体(C1)（ATCC #21597-B1）以 5×10^6 的效价加入. 然后让该混合物在 37℃温度下维持 18 分钟，期间把感染细胞倾入到冰块上以把溶液的温度降低至 15℃以下. 然后在冷冻离心机中收集被感染细胞，将其以原始体积的 1/300 悬浮于 0.1M 的磷酸缓冲液中，所述

缓冲液的 pH 为 6.1，并含有 5×10^{-3}M 的二硫苏糖醇和 10μg DNA 酶。这些细胞将溶解释放出嗜菌体和细胞溶素酶。以 100,000Xg 离心 5 小时以除去大部分细胞碎片和嗜菌体，把酶溶液分成等份试样，然后测试其溶解 A 组链球菌的能力。

测定许多酶的单位/毫升数目使其成为酶的最高稀释的倒数，从而在 15 分钟内把 A 组链球菌的悬浮液的 OD650 从 0.3 降低至 0.15 。在典型的酶制剂中，在单个 12 升批量中生成 4×10^{5} 至 4×10^{6} 单位。

根据所需要的培养时间，酶的应用需要最少量的细胞溶素酶单位。用含有稳定缓冲物质的合适调节剂稀释酶，所述调节剂能使酶稳定并发挥最大酶活性。优选的方案为应用冷冻干燥的细胞溶素酶。所述稳定缓冲物质可以含有还原剂，该还原剂可以是二硫苏糖醇，浓度为 0.001 M－1.0 M，优选 0.005 M。所述稳定的缓冲物质可以含有金属螯合剂，其可以是 0.00001M 至 1.0M、优选 0.005M 浓度的乙二胺四乙酸二钠盐。该稳定缓冲物质可以含有柠檬酸磷酸盐缓冲剂，浓度为 0.001 M－1.0 M，优选 0.05 M。所述稳定的缓冲剂的 pH 值范围是约 4.0-8.0、优选 6.1。稳定的缓冲物质可以含有作为防腐剂的杀菌剂或抗菌剂。这样的防腐剂可以是叠氮化钠，其浓度为 0.001%至 0.1%，优选 0.02%。

用于产生细胞溶素的嗜菌体原料的制备可以按照上述在细胞溶素酶制备中用于感染嗜菌体和 C 组链球菌的相同方法进行。然而，此制备方法并不把被感染细胞倾入到冰上，在 37℃温度下培养的时间共持续 1 小时，以使嗜菌体和酶在总体积中溶解和释放。为了把嗜菌体用于随后的细胞溶素生成，必须灭活残余酶，或者将这些酶除去以防止从没有 C 组噬菌体的细胞而不是嗜菌体感染的细胞中溶解。

实施例 2

按照实施例 1 制备的酶稀释至浓度为 100 单位/毫升的缓冲液中，所述缓冲液的组成为：0.05M 柠檬酸盐磷酸盐缓冲液（其 pH 为 6.1 并含有 0.1%的兔免疫球蛋白、0.005M（亚乙基二硝基）四乙酸二钠盐（EDTA）、0.005M 二硫苏糖醇、0.02% 叠氮化钠、0.01% N-乙酰基葡糖胺。把用 A 组链球菌抗体标记的胶体金溶胶（OD_{520}1.5）悬浮于 0.02 M Tris 缓冲液（其 pH 为 8.2，含有 1.0%牛血清白蛋白、0.02%叠氮化钠、300K 单位肝素）中，将 1 份此悬浮液加入到 3 份酶试剂中，混合，

并通过 0.22 微米滤器过滤，每试管中含有 200 微升等份样品，进行冷冻干燥。该冷冻干燥的酶在升高的温度下（即 45℃）能稳定短时间（2 周），在室温下能长期（大于 1 年）保持稳定。

实施例 3

方法

1. 把 A 组链球菌株 S43/192/39R（抗链霉素型）培养 1 天（从冷冻血肉汤中）；将 500μl 置于 50ml 含有 1% 酵母提取物和 100μl 链霉素/ml 的 Todd Hewitt(TH)肉汤中。

2. 培养至 OD_{650} 为 0.59。

3. 在 3000rpm 下离心 15 分钟以沉淀细菌。

4. 把这些生物体重新悬浮于 1ml 体积 TH w/o 抗生素（3×10^5/100μl，由平板计数确定）。

5. 把 0.5 这些浓度的细胞加入到 1.5ml pH 6.1 的磷酸缓冲液中作为对照。

6. 在给药至小鼠前 5 分钟，把 0.5ml 浓缩的细胞悬浮液与 0.5ml 嗜菌体细胞溶素在 pH6.1 磷酸缓冲液中的溶液混合，其中所述嗜菌体细胞溶素溶液被预先稀释至 10,000 单位/毫升。

把 5 只接受 60μl “对照”溶液的小鼠分开，等量口腔用药和鼻腔用药。

把 5 只接受 60μl 细胞溶素和细菌混合物的小鼠分开，等量口腔用药和鼻腔用药。

在 5% 绵羊血、脲蛋白胨琼脂培养皿上进行喉擦洗，所述培养皿含有 500μg/ml 链霉素。在 37℃温度下培养表面皿过夜。

得到下列结果：

	7/22	7/23	7/24	7/28
	1 天	2 天	3 天	7 天
	菌落形成单位			
细胞溶素				
L1	0	0	0	0
L2	0	0	0	0
L3	0	0	0	0
L4	0	1	0	0
L5	0	0	1	0
对照				
C1	26	14	7	0
C2	>400	17	100	83
C3	9	0	15	0
C4	>400	>400	>400	220
C5	2	2	30	0

这些结果显示：在此模型系统中，嗜菌体细胞溶素与 A 组链球菌仅仅接触 5 分钟就能防止所述链球菌集居于小鼠上呼吸道。

实施例 4

为了复制可能发生的情况，在链球菌进入环境、动物、细菌前把细胞溶素置于口腔（即通过锭剂释放），然后按照实施例 3（1－5）同样的方式制备细胞溶素，但在本实验中，动物作如下处理：

细胞溶素组：让 5 只小鼠接受 25μl 细胞溶素，然后立即让其接受 50μl 细菌悬浮液。

对照组：让 5 只小鼠接受 25μl 缓冲液，然后立即让其接受 50μl 细菌悬浮液。

24 小时

细胞溶素

L6 −

L7 −

L8 −

L9 −

L10 −

对照组

C6 −

C7 +

C8 −

C9 +

C10 +

结果：当加入链球菌时，对于口腔中含有细胞溶素的动物有 0/5 的比例被菌集，而对于接受缓冲液的动物有 3/5 的比例被菌集链球菌。因此，如果在加入 A 组链球菌之前存在细胞溶素可以防止菌集。

实施例 5

在 37℃下，在 Todd Hewitt 培养基中培养 AM 组 6 型链球菌过夜。用无菌细胞溶素缓冲液（50ml 磷酸盐缓冲液，pH6.1）洗涤生物体 1 次。然后把细胞沉淀物悬浮于 5ml 同样的缓冲液中。

在含有 5mM DTT 的细胞溶素缓冲液中，把嗜菌体细胞溶素稀释至两倍合适的单位，然后把混合物进行无菌过滤。

把 1.0ml 细菌悬浮液加入到 1.0 ml 合适的细胞溶素稀释液中，然后在 37℃温度下培养该混合物。以一定的时间间隔除去样品，进行适当稀释，将其置于血血琼脂培养皿中以测定细菌数目。

把由 1.0ml 细菌悬浮液组成的对照样品单独加入到 1.0 ml 细胞溶素缓冲液中。除去等分样品，然后在 10 倍稀释液中稀释，把等份样品置于血琼脂培养皿中以测定细菌数目。

结果		含细胞溶素的细菌数				
细胞溶素单位	起始数目	5秒	30秒	60秒	5分钟	10分钟
1000	5×10^6	0	0	0	0	0(平均2 exp)
100	8.6×10^6	1530	1196	771	64	6(平均4 exp)
10	9.8×10^6	>3000	>3000	>3000	>3000	>3000(平均3 exp)

根据上述教导可以对本发明作出许多改进和变动. 因此应该理解: 本发明的范围由权利要求书确定, 而不限于说明书具体说明的方案.