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Research Article

Controlled-Release Delivery Systems for Proteins and Peptides: A Third Generation Recombinant Vaccine-Delivery System for Single-dose Subcutaneous Administration

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Abstract

The objective was to develop Microsphere-based Delivery Systems (MDS) for controlled-release delivery of recombinant anthrax vaccine *via* single-dose subcutaneous administration. Microsphere-based Recombinant Protective Antigen (RPA) delivery systems for subcutaneous immunization were successfully developed at PolyMicrospheres, wherein the MDS formulations, on a single-subcutaneous dose, produced extremely high antibody titers (over 150000 in mice after 84 days and over 85000 in mice after 106 days of immunization) compared to the aqueous RPA vaccine system (at 8100). Mice immunized with selected MDS systems were challenged with anthrax-toxin. The MDS systems, on a single-subcutaneous dose, showed 100% protection against anthrax-toxin challenge in mice, compared to <13% protected by the aqueous RPA vaccine system and 0% in the non-immunized control group. Also, rabbits immunized with selected MDS systems were challenged with live anthrax-spores. The MDS systems, on a single-subcutaneous dose, showed 100% protection against anthrax-spore challenge in rabbits, compared to <17% protected by the aqueous RPA vaccine system and 0% in the non-immunized control group.

Keywords: Microsphere-based delivery systems; Controlled-release; Recombinant anthrax vaccine

Objectives

The general objective of the work was to develop and evaluate microsphere-based antigen delivery systems to enhance the efficacy of the RPA vaccine *via* single-dose subcutaneous immunization. To demonstrate that effective protection against anthrax can be achieved by a single-dose vaccination, PolyMicrospheres developed and evaluated novel antigen-adjuvant delivery systems. The efficacy of vaccination can be considerably improved not only by incorporating the antigen in a matrix, but also by incorporating potent adjuvants in the matrix to provide long-term delivery of antigen together with an adjuvant for further potentiation of the immune response (Figure 1).

Depending on the composition, the matrix delivery system releases the incorporated RPA/adjuvant at many distinct time points, stimulating primary and many booster responses for better immunity and protection. The controlled-release kinetics and the consequent multiple antibody peaks assure a long-persisting immunity and protection for at least one year.

Background and Significance

The possibility of biological warfare and bioterrorism is an increasing threat in today's world. Among these weapons, anthrax has become the most prominent threat. It is only prudent to take steps to minimize the damage from such an act of bioterrorism. One of the most effective precautions will be a single-dose subcutaneous immunization with an effective vaccine delivery system.

The *Bacillus anthracis* organism can be very easily produced in bulk quantities and disseminated as stable, long-lasting spores which can infect a large population *via* inhalation or contact [1,2]. The most serious route of infection is pulmonary. The spores germinate and quickly disseminate in the hilar and tracheal lymph nodes; the ensuing bacteremia produces over 80% mortality within a short period. A recombinant PA vaccine has been developed, and immunization with this protein has offered significant protection against pulmonary anthrax [3-7].

In a combat situation, logistics of vaccine administration, compliance, and time are of the essence. Vaccination with the first generation of anthrax vaccines, after the initial injection, requires five parenteral booster doses in 18 months. Furthermore, side effects occur, which can range from local soreness to fever and illness, with increased chances of occurrence after a booster injection. The second-generation vaccine, Recombinant Protective Antigen (RPA) with alum adjuvant, requires three or four vaccinations over an 18 month period. Thus, there is a need for an effective single-dose vaccine delivery system which offers long-term protection without multiple boosters. In this applied research, we developed and evaluated microsphere-based antigen-adjuvant delivery systems to enhance the efficacy of RPA vaccine *via* a single-dose subcutaneous administration. This platform technology could be utilized not only

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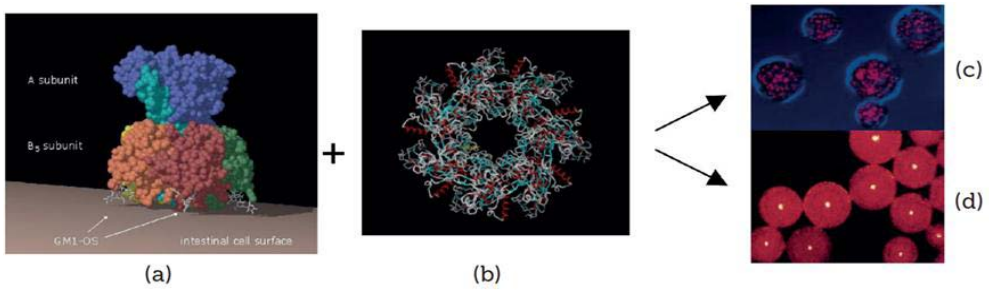

Figure 1: RPA (a) Bound to an adjuvant (b) Incorporated into heterogeneous microspheres (c) Or homogeneous microspheres (d) With controlled-release kinetics over a period of 15 weeks stimulating antibody response at many distinct time points. The released RPA/adjuvant binds to the cell surface by the B submit of the adjuvant. After entering the antibody producing cells, a long-lasting and enhanced immune response and protection is stimulated.

against inhalation anthrax but also against other microbes.

Controlled release of antigens from polymer microparticles has been of particular interest in the development of vaccine delivery systems [8-10]. The efficacy of vaccination can be improved not only by incorporating the antigen in the polymer matrix, but also by incorporating potent adjuvants in the matrix to provide long-term delivery of antigen together with a vaccine-adjuvant for further potentiation of the immune response. Many modern vaccines are composed of highly purified or recombinant proteins or synthetic peptides. The use of potent adjuvants to enhance immune response to these antigens is an attractive method for improving their immunogenicity. Such adjuvants include CpG motifs, lipopolysaccharide, polyIC and monophosphoryl lipid A [11,12]. Other potent adjuvants include LTR72 and LTK63 [13,14]. The biological activity of LTR72 and LTK63 is by cytokine-stimulated robust enhancement of both the humoral and the cellular immune response.

Materials and Methods

The MDS were designed and developed as follows: In order to achieve full protection by a single-dose subcutaneous immunization, the delivery system needed to be optimally designed using appropriate microencapsulation methods and finely tuned release kinetics. An ideal combination consists of the following: the second generation RPA, a potent mucosal adjuvant (Adj), RPA-Adj ratio, (lactide/glycolide) ratio for the right half life, microsphere particle diameter, the proper drug load, and the process parameters to achieve stability and integrity of the conformation of RPA. Because of these reasons and due to the extremely high cost of RPA, it was essential to develop, evaluate and refine the delivery systems in multiple stages. With PolyMicrospheres expertise and experience in this area, we were able to develop, test and refine the formulations as we progressed through each stage with the results from the animal studies.

The novel RPA/Adj delivery systems were designed to provide controlled and pulsed-release delivery of the recombinant anthrax vaccine equivalent to multiple immunizations. A potent adjuvant such as LTK-63 was also incorporated into the microsphere matrices to provide a long-term delivery of a vaccine adjuvant for further potentiation of the immune system. In these MDS, both the RPA and the Adj are incorporated into the same microsphere matrices. We developed heterogeneous (Type I) and homogeneous (Type II) formulations to achieve different release rates. Depending on the optimal combination of the RPA/Adj in the polymer matrix, the molar ratio of lactide-glycolide, drug loading, particle diameter, and the microencapsulation methods and process techniques, the content is released in a controlled manner at multiple time-points stimulating antibody peaks several weeks apart, thereby stimulating a long-lasting (at least 1 year) immunity and protection. The delivery systems were designed such that the antigen can reach the mucosal antigen processing cells in its native conformation for induction of an effective protective immunity.

Preparation of the MDS formulations

Polymers: Poly(dl-lactide-co-glycolide) and Poly(dl-lactide)

Microsphere Mean Diameter range: 4-33 μM

RPA of anthrax (from List Biological) Loading: 0.5-1%

Adjuvant (LTK63 from Chiron) Loading: 0.02-0.19%

MDS formulations were prepared using established protocols currently in use at PolyMicrospheres [15]. A modified complex coacervation process was used. Processes include heterogeneous (Process I leading to Type I products) and homogeneous (Process II leading to Type II products) water-in-oil primary emulsions leading to different matrix-formulations to release the antigen and adjuvant at different rates.

Analytical methods for the characterization

The MDS formulations developed were characterized as to mean particle diameter, size distribution, antigen and adjuvant loadings using established protocols currently in use at PolyMicrospheres [15].

a) Particle size and size distribution analysis using Shimadzu Laser Diffraction Particle Size Analyzer and/or Coulter Multi-angle Sub-Micron Particle size Analyzer.

b) Particle aggregation, uniformity, shape and surface morphology analysis using a Nikon-Diaphot high resolution inverted microscope.

c) Measurement of antigen/adjuvant loadings in the microparticle delivery systems: A known weight of the microparticle sample was dissolved in a suitable volume of solvent and extracted with a known volume of PBS. The concentration of protein was determined using a Bio-Rad Protein Assay Kit.

Protocols for vaccination of mice and efficacy

Selected formulations were tested for their ability to induce a systemic antibody response in mice following a single-dose

subcutaneous administration. The immune response was assessed by an ELISA assay of periodic test bleeds for anti-RPA antibodies.

Mouse immunization protocol: Groups of 8 AJ mice were immunized with 3.3-3.8 mg each of MDS incorporated with both RPA and Adj by a single-dose subcutaneous administration. Control groups include aqueous RPA system and non-immunized control. Mice were bled from the retro-orbital sinus under light anesthesia over a 15 week period after immunization. Serum was prepared and assayed for anti-PA antibodies by ELISA.

ELISA assay for mouse anti-PA antibodies: Individual serum samples bled at various time points (30, 50, 84 and 106 days after immunization) were assayed for anti-PA immunoglobulins using standard ELISA protocols. Horse radish peroxidase-labeled anti-mouse antibody directed against IgG was used. The amount produced was determined spectrophotometrically. A standard curve was prepared using known amounts of purified mouse anti-PA antibodies, obtained from USAMRIID as a positive control, and the amount of anti-PA antibodies in the samples was determined.

Anthrax-toxin challenge studies in mice: Selective MDS formulations were tested for their efficacy in inducing protection against a lethal challenge of anthrax toxin. Control groups include an aqueous RPA system and a non-immunized group. Mice were bled as described above from the retro-orbital sinus for a period of 15 weeks. The serum was tested for antibody titers to assure that animals receiving the vaccine have responded. The anthrax-toxin challenge was performed after 110 days of immunization. This challenge consisted of intravenous injection of a mixture of lethal factor (1.5mg/kg) and protective antigen (3mg/kg) in a combination equivalent of approximately five LD_{50} in non-immunized mice. On day 42 after the toxin challenge, the experiments were terminated.

Protocols for vaccination of rabbits and efficacy

Rabbits were immunized with selected MDS formulations by a single-dose (or two-dose) subcutaneous administration and monitored for antibody response over a 6-week period following the immunization. Control groups include aqueous RPA system and a non-immunized control.

Rabbit immunization protocol: Groups of 6-8 New Zealand White rabbits were immunized with 12-14 mg (for MDS-E to MDS-G) and 6-8 mg (for MDS-H & MDS-I) each of MDS incorporated with both RPA and Adj by a single-dose subcutaneous administration. For additional studies *via* two-dose administration, the second dose was given at the same concentration after three weeks of the first immunization. The rabbits were bled from the ear vein 18, 31, and 45 days after immunization. Serum was prepared and assayed for anti-PA antibodies by ELISA.

ELISA assay for rabbit anti-PA antibodies: The ELISA assay for the rabbit experiments was similar to that described above for measuring mouse anti-PA antibodies except that horse radish peroxidase-labeled anti-rabbit Ig antibodies were used.

Anthrax-spore challenge studies in rabbits: The immunized rabbits were challenged with a lethal aerosol exposure of live B. Anthracis (Ames) spores and monitored for survival. All animals were aerosol-challenged on Study Day 0 with a target dose of 200 LD_{50}. Animals were observed twice daily for 14 days post-challenge for clinical signs of disease including but not limited to lethargy and respiratory distress. At the end of the 14-day observation period, the surviving rabbits were euthanized and the carcasses autoclaved and then incinerated. All B. anthracis challenges were performed in a BL-3 containment laboratory.

Protocols for the safety and histopathology studies

Four to six weeks after immunization of selected MDS formulations at the dose used in the immunization protocol, five mice/rabbits at each time point were sacrificed for complete organ histopathology to rule out toxic side effects.

At these time points, blood samples were collected prior to sacrificing the mice/rabbits for routine serum chemistry, including liver and kidney function tests, creatinine kinase, and complete hematology parameter determinations. **Evaluation criteria:** Compare the histopathology and serum chemistry including kidney and liver function tests with normal untreated animals.

Results and Discussion

The RPA-adjuvant delivery systems were designed, developed and crafted at PolyMicrospheres in many stages. Each stage consisted of a group of optimal formulations with various diameters, polymer matrices, and drug loadings to provide controlled and pulsed-release of the anthrax vaccine. As the formulations were developed, they were tested systematically in mice (as described above). As we progressed, based on the results, selections were made to further test the formulations in rabbits (as described above). The best formulations with high immunogenicity in rabbits were used to immunize rabbits and challenged against live anthrax spores.

The antibody titers of immunity stimulated with MDS were determined in mice. Based on the results, formulations were refined, redesigned, and the process conditions were altered to obtain formulations with the desired integrity, antigen/adjuvant content, and release kinetics required to stimulate full protection against the anthrax-toxin and live anthrax-spore challenge. Development and evaluation were done side-by-side and stage-by-stage. We developed dozens of MDS formulations using established protocols currently in practice at PolyMicrospheres to achieve the ideal release rates. Only selective MDS formulations with significant results are discussed here.

Development and optimization of MDS for RPA vaccine

Significant effort was focused on the design and development of the RPA- and Adj- incorporated MDS, wherein both the RPA and the Adj were incorporated into the same microspheres (Table 1).

Studies on immunization of mice with MDS *via* single-dose subcutaneous administration

We have immunized mice via single-dose subcutaneous administration with the MDS systems. The MDS products were tested for their efficacy to induce an antibody response in mice. Figure 2 shows the antibody (IgG) response in mice immunized with selected MDS systems via single-dose subcutaneous administration. Mice immunized with selected MDS products showing high antibody titers were challenged with anthrax toxin 110 days after immunization, also shown in Figure 2.

Table 1: MDS incorporated with both RPA and ADJ in the same microspheres.

MDS Product code	Mean Diameter	Drug Loading		Type, inner-phase (see Methods)
		RPA	Adj	
MDS for Mice studies:				
MDS-A	9 μM	0.96%	0.02%	I, heterogeneous
MDS-B	16 μM	0.81%	0.04%	II, homogeneous
MDS-C	12 μM	0.88%	0.08%	I, heterogeneous
MDS-D	33 μM	0.96%	0.08%	II, homogeneous
MDS for Rabbit studies:				
MDS- E	4 μM	0.49%	0.13%	I, heterogeneous
MDS-F	8 μM	0.48%	0.13%	I, heterogeneous
MDS-G	6 μM	0.63%	0.13%	I, heterogeneous
MDS-H	9 μM	0.93%	0.19%	I, heterogeneous
MDS-I	16 μM	0.92%	0.19%	II, homogeneous

Our subcutaneously-delivered MDS-C and MDS-D showed extremely high IgG titers throughout the 106-day testing period, exhibiting a 4-5x increase over the parenterally-delivered positive control (and a 10-13x increase over the aqueous RPA system). Since MDS-C and MDS-D exhibited over 140,000 IgG titers 50 days after immunization, it is very likely that the MDS immunized mice were already fully protected in 50 days after single-dose subcutaneous immunization. The IgG titers induced by these MDS systems remained high throughout the testing period of 106 days, indicating a continuous controlled (or pulsatile) release of the RPA and Adj over 15 weeks.

In the anthrax-toxin challenge studies, all control (non-immunized) mice died with the first three days, demonstrating that the challenge was correctly administered to the mice. The median survival time of mice immunized with aqueous RPA system was less than 10 days (7 died in under 6 days, and one survived), showing <13% protection against the toxin challenge. But mice immunized with MDS-C and MDS-D had all survivors and showed 100% protection against the anthrax toxin challenge on the 42nd day when the experiments were terminated.

These microsphere-based vaccine delivery systems afforded good protection against anthrax toxin while the aqueous vaccine system did not.

Studies on immunization of rabbits with MDS *via* single-dose subcutaneous administration followed by anthrax-spore challenge

We have evaluated the efficacy of selected MDS formulations by a single-dose subcutaneous administration to rabbits. Rabbits immunized with MDS products showing high antibody titers were challenged with live anthrax-spores after 62 days of immunization. Figure 3 includes anthrax-spore challenge studies on selected MDS systems.

These MDS systems all produced high antibody titers 45 days after immunization and also offered 100% protection against live anthrax spores. Therefore the MDS immunized rabbits were already fully protected by at least 45 days following the immunization. MDS-E to G exhibited antibody titers in rabbits at least a 10-fold increase (on 45 days) over the aqueous RPA system. The IgG titers induced by the MDS systems remained high throughout the testing period, indicating a continuous controlled (or pulsatile) release of the RPA and Adj over the testing period of 1.5 months.

These results clearly indicate that new Formulations MDS-E, F, G and I afforded a 100% protection against inhalation anthrax by a single-dose subcutaneous administration, unlike the aqueous RPA system (<17%). Preliminary histopathology studies indicate that the heart, lungs, liver, spleen, kidneys and small intestine are normal in rabbits immunized with Formulations MDS-E and F and did not show any toxic effects.

These results indicate that we have successfully developed a viable single-dose subcutaneous delivery system for recombinant anthrax vaccine. Microsphere-based subcutaneous RPA/Adj delivery system inoculated to mice/rabbits substantially augmented the RPA-

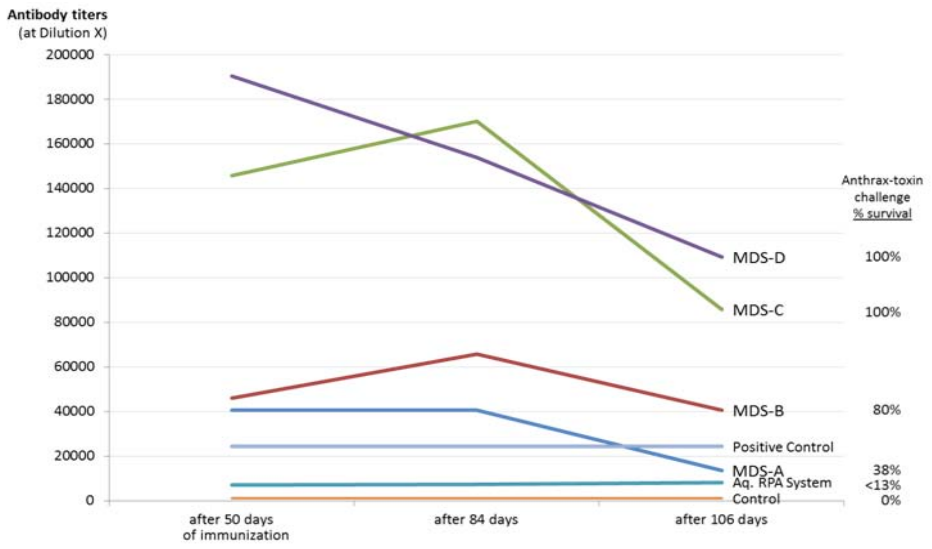

Figure 2: Immune Response to selected MDS systems in Mice *via* single-dose Subcutaneous Immunization: Antibody (IgG) Titers.

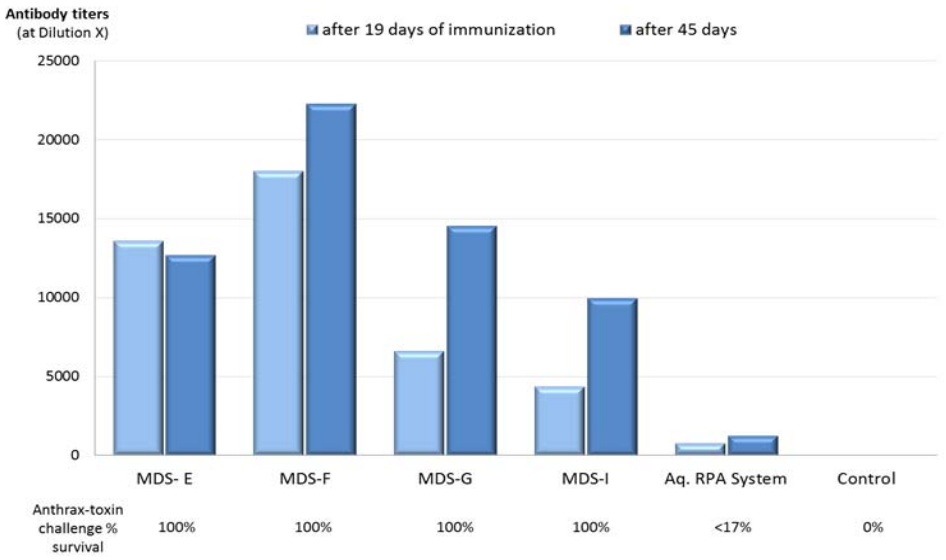

Figure 3: Efficacy of MDS formulations by a single-dose administration to rabbits followed by live anthrax-spore challenge.

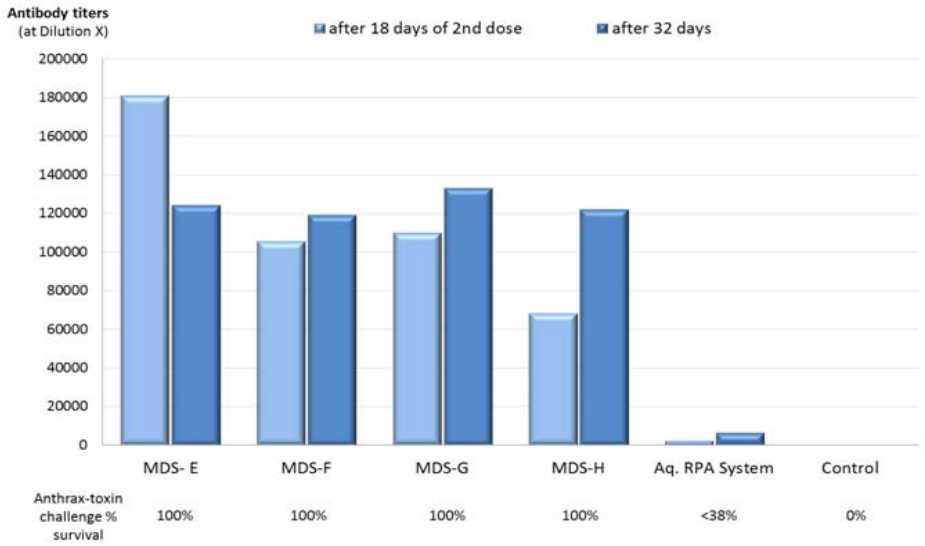

Figure 4: Efficacy of MDS formulations by a two-dose administration to rabbits.

specific ELISA IgG titers compared to the aqueous RPA system titers. Unlike the aqueous RPA system, mice vaccinated with a single-dose subcutaneous administration of MDS system resisted challenge to anthrax toxin. Also, MDS-E, F, G and I afforded a 100% protection of rabbits against inhalation anthrax by a single-dose subcutaneous administration, unlike the aqueous RPA system.

Additional studies

Immunization of rabbits with MDS *via* two-dose subcutaneous administration: Although we obtained 100% protection against anthrax-spores with a single-dose, we evaluated the efficacy of selected MDS formulations by a two-dose subcutaneous administration to rabbits for the following reasons:

(i) To compare the efficacy of a single-dose and two-doses.

(ii) To see the effect of the second-dose on the magnitude of rise of the antibody titers.

Even though we were confident that the MDS systems would offer 100% protection with two-doses since the single-dose already offered 100% protection, we challenged the rabbits immunized with two-doses of the MDS systems against live anthrax spores after 41 days of immunization. The results are shown in Figure 4.

These MDS systems all produced >100,000 antibody titers 32 days after the second-dose administration, exhibiting a 17-fold increase over the aqueous RPA system. In addition, these MDS systems offered a 100% protection against live anthrax spores. Therefore, these MDS immunized rabbits were already fully protected by 32 days following the immunization. The IgG titers induced by the MDS systems remained high throughout the testing period, indicating a continuous controlled (or pulsatile) release of the RPA and Adj

over the testing period. Because of the extremely high antibody titers obtained, the two-dose subcutaneous immunization is most likely to offer full protection for more than a year.

Histopathology studies on immunized rabbits

Preliminary histopathology studies indicate that the heart, lungs, liver, spleen, kidneys and small intestine are normal in rabbits immunized with the delivery systems MDS-E and MDS-H, and they did not show any toxic effects.

Conclusion

In the review article [8] on "New advances in microsphere-based single-dose vaccines", Hanes, Cleland and Langer wrote in their conclusion, "Due to their many unique properties (including their ability to provide a long-term depot for antigen, to target macrophage phagocytosis leading to enhanced humoral and cellular immunity and to their potential stability under warm, dry conditions) microsphere-based vaccines may eventually meet the challenge of providing immunity in a single dose, a goal considered the 'holy-grail' of vaccine delivery".

In this applied research, PolyMicrospheres has successfully developed a viable single-dose subcutaneous microsphere-based delivery system offering an effective delivery of recombinant anthrax vaccine. MDS-C and MDS-D systems delivered over 85000 antibody titers in mice (after 106 days), compared to less than 8100 for the aqueous RPA system. Also, MDS-E to G exhibited antibody titers in rabbits at least a 10-fold increase (after 45 days) over the aqueous RPA system. In addition, the novel MDS showed 100% protection in mice against lethal anthrax toxin challenge, while the aqueous RPA system was ineffective. Also, MDS afforded a 100% protection of rabbits against inhalation anthrax by a single-dose subcutaneous administration, unlike the aqueous RPA system. Preliminary histopathology studies of the MDS did not show any toxic effects. In addition, it is very likely that the MDS immunized rabbits were already fully protected in 45 days after the immunization.

The platform technology of this microsphere-based single-dose subcutaneous delivery system is also suitable for other human and veterinary vaccines including simultaneous delivery of multiple immunogens.

Impact of this project on the armed forces and civilian population

The second-generation anthrax vaccine (RPA + alumina) currently in the field requires three-four doses spread over 18 months. Immunization with a single-dose of the novel MDS formulation considerably reduces the time required to attain combat readiness of the Armed Forces, minimizes side-effects, reduces the number of repeated-trips to the medical facility, and simplifies the logistics of the immunization of large numbers of military personnel, plus the added benefit of reducing the cost of the immunization.

The civilian population would be ready in shorter time with the single-dose immunization in response to bioterrorism or natural outbreaks. The single-dose administration simplifies the logistics of mass immunization and would increase the availability of the vaccine. It could be done easily and quickly, reducing the cost of the vaccine and its administration.

References

1. Inglesby TV, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Friedlander AM, *et al*. Anthrax as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. JAMA. 1999; 281: 1735-1745.
2. Scorpio A, Blank TE, Day WA, Chabot DJ. Anthrax vaccines: Pasteur to the present. Cell Mol Life Sci. 2006; 63: 2237-2248.
3. Gu ML, Leppla SH, Klinman DM. Protection against anthrax toxin by vaccination with a DNA plasmid encoding anthrax protective antigen. Vaccine. 1999; 17: 340-344.
4. Demicheli V, Rivetti D, Deeks JJ, Jefferson T, Pratt M. The effectiveness and safety of vaccines against human anthrax: a systematic review. Vaccine. 1998; 16: 880-884.
5. Friedlander AM, Pittman PR, Parker GW. Anthrax vaccine: evidence for safety and efficacy against inhalational anthrax. JAMA. 1999; 282: 2104-2106.
6. Friedlander AM, Little SF. Advances in the development of next-generation anthrax vaccines. Vaccine. 2009, 27, D28-D32.
7. Cybulski RJ, Sanz P, O'Brien AD. Anthrax vaccination strategies. Mol Aspects Med. 2009; 30: 490-502.
8. Hans J, Cleland JL, Langer R. New advances in microsphere-based single-dose vaccines. Adv Drug Deliv Rev. 1997; 28: 97-119.
9. Igartua M, Hernandez RM, Esquisabel A, Gascon AR, Calvo MB, Pedraz JL. Enhanced immune response after subcutaneous and oral immunization with biodegradable PLGA microspheres. J Control Release. 1998; 56: 63-73.
10. Yan C, Rill WL, Malli R, Hewetson J, Naseem H, Tammariello R, *et al*. Intranasal stimulation of long-lasting immunity against aerosol ricin challenge with ricin toxoid vaccine encapsulated in polymeric microspheres. Vaccine. 1996; 14: 1031-1038.
11. Klinman DM, Klaschik S, Tomaru K, Shirota H, Tross D, Ikeuchi H. Immunostimulatory CpG oligonucleotides: Effect on gene expression and utility as vaccine adjuvants. Vaccine. 2010; 28: 1919-1923.
12. Baldridge JR, Yorgensen Y, Ward JR, Ulrich JT. Monoposphoryl lipid A enhances mucosal and systemic immunity to vaccine antigens following intranasal administration. Vaccine. 2000; 18: 2416-25.
13. Giuliani MM, Del Giudice G, Gianelli V, Dougan G, Douce G, Rappuoli R, *et al*. Mucosal adjuvanticity and immunogenicity of LTR72, a novel mutant of Eschericia coli heat-labile enterotoxin with partial knockout of ADP-rybosyltransferase activity. J Exp Med. 1998; 187: 1123-32.
14. Kende M, Del Giudice G, Rivera N, Hewetson J. Enhancement of intranasal vaccination in mice with deglycosylated chain A ricin by LTR72, a novel mucosal adjuvant. Vaccine. 2006; 24: 2213-2221.
15. March KL, Mohanraj S, Ho PP, Wilensky RL, Hathaway DR. Biodegradable microspheres containing a colchicine analogue inhibit DNA synthesis in vascular smooth muscle cells. Circulation. 1994; 89: 1929-1933.

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