Краткие сообщения
Short communications

РАЗРАБОТКА ВАКЦИННОГО АДЪЮВАНТА НА ОСНОВЕ СКВАЛЕНА И ИЗУЧЕНИЕ ЕГО АДЪЮВАНТНЫХ СВОЙСТВ

Волосникова Е.А., Щербаков Д.Н., Ермолаев В.В., Волкова Н.В., Каплина О.Н., Боргоякова М.Б., Даниленко Е.Д.

ФБУН «Государственный научный центр вирусологии и биотехнологии "Вектор"» Роспотребнадзора, п. Кольцово, Новосибирская обл., Россия

Резюме. Использование современных субъединичных вакцин предполагает введение в их состав адъювантов. В настоящее время активно ведется поиск новых и усовершенствование существующих адъювантных систем. Адъюванты на основе сквалена известны и разрешены в ряде стран для клинического применения в составе вакцин против гриппа. Наша работа посвящена разработке адъюватной композиции, содержащей в своем составе сквален. Полученные нами адъювантные композиции представляли собой масляную эмульсию, содержащую гидрофильную и гидрофобную фазу. Стабильности эмульсии добивались путем обработки ее ультразвуком с частотой 22 кГц. Оценку размеров частиц полученных эмульсий проводили с помощью электронного микроскопа. Показано, что размер частиц большинства частиц (84%) составил от 50 до 80 нм. Оценку адъювантной активности проводили на 100 самцах мышей линии BALB/c массой 16-18 г. Для оценки гуморального иммунного ответа иммунизацию проводили двукратно с интервалом 14 суток, внутримышечной инъекцией объемом 200 мкл на животное. В качестве антигена использовали рецептор-связывающий домен поверхностного белка коронавируса SARS-CoV-2 (вариант B.1.617.2 (Delta)) либо овальбумин из куриных яиц. Рецептор-связывающий домен поверхностного белка коронавируса SARS-CoV-2 вводили в дозе 50 мкг на животное, овальбумин — 1 и 5 мкг на животное. В качестве положительного контроля использовали антиген с гидроксидом алюминия. В качестве отрицательного контроля — физиологический раствор. Эффективность полученных адъювантов определяли измерением титров специфических антител в сыворотках мышей методом ИФА с использованием рекомбинантного рецептор-связывающего домена поверхностного белка коронавируса SARS-CoV-2 (вариант B.1.617.2 (Delta)) либо овальбумин из куриных яиц. В ходе работы показано, что использование адъювантов на основе сквалена позволило увеличить иммуногенность антигенов. В случае с рецептор-связывающим доменом поверхностного белка коронавируса SARS-CoV-2 средние титры специфических антител в опытной группе в 4 раза превышали титры контрольной группы, иммунизированной антигеном с гидроокисью алюминия. Повышение иммуногенности антигена с добавлением сквалена наблюдали

Адрес для переписки:

Волосникова Екатерина Александровна
ФБУН «Государственный научный центр вирусологии
и биотехнологии "Вектор"» Роспотребнадзора
630559, Россия, Новосибирская обл., п. Кольцово.
Тел.: 8 (383) 363-80-14.
Факс: 8 (383) 363-80-16.
E-mail: volosnikova_ea@vector.nsc.ru, kulenok84@mail.ru

Address for correspondence:

Ekaterina A. Volosnikova
State Research Center of Virology and Biotechnology "Vector"
Koltsovo, Novosibirsk Region
630559 Russian Federation
Phone: +7 (383) 363-80-14.
Fax: +7 (383) 363-80-16.
E-mail: volosnikova_ea@vector.nsc.ru, kulenok84@mail.ru

в опытной группе наблюдали и в случае с овальбумином. Таким образом, показано, что разработанная адъювантная система на основе сквалена является альтернативой традиционным адъювантам на основе солей алюминия.

Ключевые слова: адъювант, сквален, иммунный ответ, S-белок, овальбумин, иммунизация

DEVELOPMENT OF A VACCINE ADJUVANT BASED ON SQUALENE AND STUDY OF ITS ADJUVANT PROPERTIES

Volosnikova E.A., Shcherbakov D.N., Ermolaev V.V., Volkova N.V., Kaplina O.N., Borgoyakova M.B., Danilenko E.D.

State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk Region, Russian Federation

Abstract. The use of modern subunit vaccines involves adjuvant introduction into their composition. Currently, the search for new and improvement of existing adjuvant systems is actively underway. Squalene-based adjuvants are well-known and approved in a number of countries for clinical use in influenza vaccines. Our study was devoted to the development of an adjuvant composition on the basis of squalene. The resulting adjuvants were composed in a form of oil emulsion containing a hydrophilic and hydrophobic phase. The stability of the emulsion was achieved by treating it with ultrasound at a frequency of 22 kHz. Particle sizes of the obtained emulsions were examined with the use of an electron microscope. The particle size was calculated to be 50-80 nm for the majority of particles (84%). Adjuvant activity was evaluated in 100 male Balb/C mice, weighing 16-18 g. To assess the humoral immune response, immunization was performed twice, with a 14-day interval, by intramuscular injection of 200 µL per animal. The receptor-binding domain (RBD) of the surface S protein of the severe acute respiratory syndrome coronavirus 2 (Delta variant (B.1.617.2)) or ovalbumin (OVA) from chicken eggs were used as antigens. RBD was administered at a dose of 50 µg/animal; OVA was administered at two doses (1 µg or 5 µg/animal). An antigen with aluminum hydroxide was used as a positive control; a saline solution was used as a negative control. The effectiveness of the obtained adjuvants was determined by measuring the titers of specific antibodies in mouse sera in ELISA assays using the recombinant RBD of SARS-CoV-2 S-protein or ovalbumin from chicken eggs. It was shown that the use of squalene-based adjuvants increased the antigens' immunogenicity. The average titers of specific antibodies against RBD in the experimental group were 4 times higher than in the group immunized with RBD adjuvanted with aluminum hydroxide. An increase in immunogenicity of the antigen adjuvanted with squalene was also observed in the experimental OVA-group. Thus, it was shown that the developed squalene-based adjuvant compositions could be an alternative to the traditional adjuvants based on aluminum salts.

Keywords: adjuvant, squalene, immune response, S protein, ovalbumin, immunization

Introduction

Subunit vaccines continue to play an important role in the vaccination to prevent infectious diseases. One of the recent examples is the WHO approval of the subunit Mosquirix vaccine against malaria [6]. Long-term studies show that subunit vaccines, while possessing an unsurpassed safety profile, ease of production and high stability, nevertheless have relatively low immunogenicity. Therefore, adjuvants are an obligatory component of the vaccine preparations based on recombinant proteins. Through the use of adjuvants, it can be possible not only to increase immunogenicity, but also to induce mucosal immunity or trigger/stimulate cellular response mechanisms [4].

The most commonly used adjuvants in vaccines nowadays are aluminum salts. Their mechanisms of action are still not completely clear [1]. One of the main versions is the induction of a local inflammatory

process at the site of vaccine administration, which can elicit the development of a pronounced local and systemic reaction [5].

In addition to aluminum compounds, among those officially approved, adjuvants based on *Quillaja saponaria* saponins, CpG and squalene are being currently used [7]. The latter, in the form of MF59 adjuvant, has been successfully used in influenza vaccines for more than 15 years, with its high efficacy and safety being confirmed [2].

The purpose of the present work was to develop an adjuvant composition based on squalene and to study its effect on the vaccination effectiveness.

Materials and methods

Adjuvant composition assembly

The formulation of adjuvant compositions was calculated for the final preparation to have squalene at the concentration of 4.3 or 8.6% of the volume. The hydrophobic phase consisted of phospholipids dissolved in squalene ($0.5 - 1\%$ of the final volume), the hydrophilic phase — the Twin 80 emulsifier dissolved in PBS (pH 7.6). The emulsion was obtained by treating the combined hydrophilic and hydrophobic phases with ultrasound with a frequency of 20-60 kHz. The resulting emulsion was sterile filtered through $0.22\ \mu m$ bacterial filters.

For the control, an incomplete adjuvant was obtained, which comprised all the components of the composition described above except squalene.

Animal immunization

Ovalbumin (OVA) from chicken eggs or the receptor-binding domain (RBD) of the surface S-protein of the severe acute respiratory syndrome coronavirus 2 (Delta variant (B.1.617.2), obtained at the SRC VB "Vector" using CHO-K1 cells, were chosen as antigens.

The study of the adjuvant properties of the drug was carried out in male Balb/C mice, weighing 18-22 g, aged 6-8 weeks, obtained from the Nursery of the SRC VB "Vector" of Rospotrebnadzor, Koltsovo, Novosibirsk Region. Animal experiments were approved by the Bioethics Committee of the State Research Center of Virology and Biotechnology "Vector" (SRC VB Vector/September 10, 2020, approved by the protocol of Bioethics Committee No. 5 as of October 1, 2020). Mice of the positive control groups were administered intramuscularly twice, with a 14-day interval, with 1 and 5 µg OVA or 50 µg RBD in a volume of 200 µL/mouse (100 µL in each hind paw).

Mice of the experimental groups were administered with OVA at the doses of 1 and 5 µg/mouse or RBD at the doses of 25 and 50 µg/mouse (in a volume of 200 µL/mouse) in combination with the obtained adjuvant composition comprising 4.3 or 8.6% squalene. The mice of the negative control group were injected with an equivalent volume of the saline solution. Mice of the comparison groups were immunized intramuscularly twice, with a 14 day interval, with 50 µg RBD mixed with aluminum hydroxide or with 1 and 5 µg OVA mixed with aluminum hydroxide or an incomplete adjuvant.

Blood sampling was performed on day 7 after the second immunization as described in [3].

To detect the titer of specific antibodies in the sera of immunized mice, the 96-well plates were coated with 100 µL of OVA (5 µg/mL) or RBD (1 µg/mL) in phosphate-buffered saline (PBS), pH 7.4-7.5. The plates were incubated for 2 hrs at 37 °C and then for 16 hrs at 4 °C. OVA (or RBD) solution was removed by shaking, followed by adding to the wells 200 µL of blocking buffer (1% BSA solution in PBS, pH 7.4, supplemented with 0.05% Twin-20). After the incubation (2 hrs at 37 °C) and three washes with the washing buffer, 100 µL of 5-fold diluted sera (from 1:200 to 1:15625) were added to the wells. A diluting solution for serums was used to control the conjugate. The plates were incubated at 310 rpm in a thermoshaker (1.5 hrs at 37 °C), and then washed four times as described above. Next, the plates were incubated with a conjugate solution of goat anti-mouse IgG-HRP antibodies (Sigma, USA), (100 µL/well), at a dilution of 1:5000 at 37°C for 1 hr and washed five times as described above. For the color reaction manifestation, a solution of chromogen-TMB (3,3',5,5'-tetramethyl-benzidine liquid substrate, slow kinetic form, for ELISA, SIGMA) was used. The plates were incubated for 30 min at RT protected from light. The reaction was stopped using a stop solution (1M sulfuric acid) in a volume of 50 µL/well. Absorbance was measured at 450 nm using a Varioskan Lux multimode microplate reader (Thermo Fisher Scientific, USA). The results were processed using GraphPad Prism 6.0 software.

Results and discussion

Currently, among the adjuvants approved for use, the leading position is still occupied by preparations based on aluminum salts. At the same time, there is a tendency to use new, more efficient and safer compositions. One of the actively developing directions is the production of adjuvant systems in the form of emulsions based on squalene [4]. The use of squalene as the basis for adjuvant compositions has undeniable advantages, such as a high safety profile and biodegradability.

To evaluate immunogenicity, a recombinant S-protein RBD of SARS-CoV-2 (Delta variant
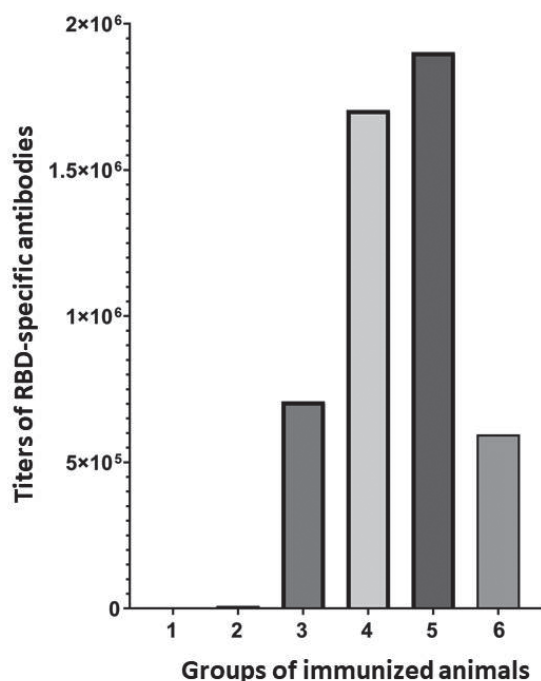
Figure 1. Reciprocal titers of RBD-specific antibodies in the blood serum of immunized BALB/c mice

Note. 1, saline solution; 2, RBD (50 μg); 3, RBD (50 μg) + aluminum hydroxide; 4, RBD (50 μg) + squalene (4.3%); 5, RBD (50 μg) + squalene (8.6%); 6, RBD (25 μg) + squalene (4.3%), respectively. The data are presented as GMTs. The graphics were made using GraphPad Prism 8.0.
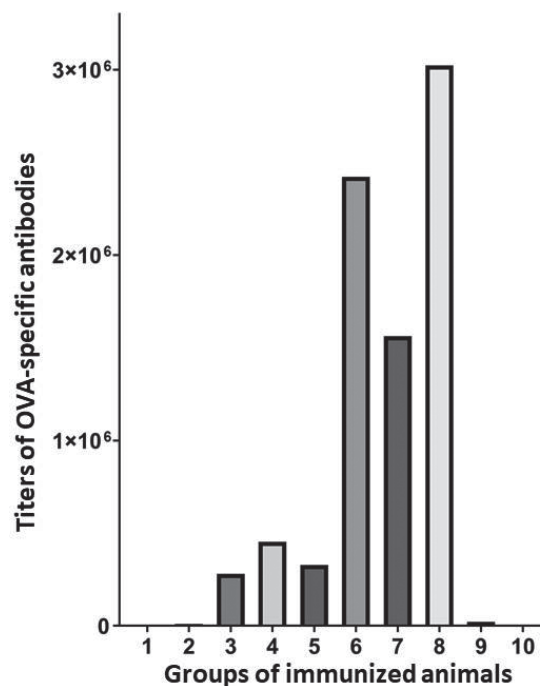

Figure 2. Reciprocal titers of OVA-specific antibodies in the blood serum of immunized BALB/c mice

Note. 1, OVA (1 μg); 2, OVA (5 μg); 3, OVA (1 μg) + aluminum hydroxide; 4, OVA (5 μg) + aluminum hydroxide; 5, OVA (1 μg) + squalene (4.3%); 6, OVA (5 μg) + squalene (4.3%); 7, OVA (1 μg) + squalene (8.6%); 8, OVA (5 μg) + squalene (8.6%); 9, OVA (1 μg) + incomplete adjuvant; 10, OVA (5 μg) + incomplete adjuvant. The data are presented as GMTs. The graphics were made using GraphPad Prism 8.0.

(B.1.617.2)) was used as one of the model antigens in our study. Analysis of mouse blood sera collected a week after the second immunization showed that the use of squalene emulsion elicited significant increase of antigens immunogenicity. The average titers of specific antibodies in the experimental group were 4 times higher than those in the group immunized with RBD with aluminum hydroxide (Figure 1). It is important to note that in the group of mice injected with a reduced dose of antigen (25 μg, group 6), the average indicator value was equivalent to the level of antibodies in the group immunized with a dose of 50 μg, but with aluminum hydroxide (group 3, Figure 1). A higher dose of squalene (8.6%) slightly increased the titer level of specific antibodies compared to its lower dose.

In many ways, similar patterns were observed in the other model, with the use of ovalbumin as an antigen. The two doses of OVA, 5 and 1 μg per mouse, were used for immunization.

Analysis of mouse blood sera after immunization showed that the use of a higher dose of adjuvant (8.6%

squalene), regardless of the antigen dose (groups 7 and 8), can significantly, almost by an order, increase the immune response compared with aluminum hydroxide. Squalene (4.3%) significantly increased the level of antibody titers in mice immunized with OVA (5 μg) (Figure 2). It is important to note that the administration of an experimental adjuvant allowed to induce high titers of specific antibodies (more than 1:1000000), even with the use of a small dose of antigen (1 μg).

Conclusion

Thus, we have obtained and evaluated an experimental adjuvant composition based on squalene. It has been shown that immunization of mice either with RBD or OVA proteins in combination with the obtained adjuvants, makes it possible to elicit a high level of humoral immune response exceeding the values achieved with the use of a classical adjuvant — aluminum hydroxide.

References

1. De Gregorio E., Caproni E., Ulmer J.B. Vaccine adjuvants: mode of action. *Front. Immunol., 2013, Vol. 4, 214.* doi:10.3389/fimmu.2013.00214

2. Durando P., Icardi G., Ansaldi F. MF59-adjuvanted vaccine: a safe and useful tool to enhance and broaden protection against seasonal influenza viruses in subjects at risk. *Expert Opin. Biol. Ther., 2010, Vol. 10, no. 4, pp. 639-651.*

3. Dyakon A.V., Hrykina I.S., Hegai A.A., Dyachenko A., Murashev A.N., Ivashev M.N. Method of blood sampling in animals. *International Journal of Applied and Fundamental Research, 2013, Vol. 11, no. 2, pp. 84-85.* (In Russ.)

4. Facciolà A., Visalli G., Laganà A., Di Pietro A. An overview of vaccine adjuvants: current evidence and future perspectives. *Vaccines, 2022, Vol. 10, no. 5, 819.* doi:10.3390/vaccines10050819.

5. Mosca F., Tritto E., Muzzi A., Monaci E., Bagnoli F., Iavarone C., O'Hagan D., Rappuoli R., de Gregorio E. Molecular and cellular signatures of human vaccine adjuvants. *Proc. Natl. Acad. Sci. USA, 2008, Vol. 105, no. 30, pp. 10501-10506.*

6. Nadeem A.Y., Shehzad A., Islam S.U., Al-Suhaimi E.A., Lee Y.S. Mosquirix™ RTS, S/AS01 Vaccine Development, Immunogenicity, and Efficacy. *Vaccines, 2022, Vol. 10, no. 5, 713.* doi:10.3390/vaccines10050713.

7. Shi S., Zhu H., Xia X., Liang Z., Ma X., Sun B. Vaccine adjuvants: Understanding the structure and mechanism of adjuvanticity. *Vaccine, 2019, Vol. 37, no. 24, pp. 3167-3178.*

Авторы:

Волосникова Е.А. *— к.б.н., ведущий научный сотрудник, заведующая лабораторией получения и анализа биосубстанций ФБУН «Государственный научный центр вирусологии и биотехнологии "Вектор"» Роспотребнадзора, п. Кольцово, Новосибирская обл., Россия*

Щербаков Д.Н. *— к.б.н., ведущий научный сотрудник ФБУН «Государственный научный центр вирусологии и биотехнологии "Вектор"» Роспотребнадзора, п. Кольцово, Новосибирская обл., Россия*

Ермолаев В.В. *— младший научный сотрудник ФБУН «Государственный научный центр вирусологии и биотехнологии "Вектор"» Роспотребнадзора, п. Кольцово, Новосибирская обл., Россия*

Волкова Н.В. *— к.б.н., младший научный сотрудник ФБУН «Государственный научный центр вирусологии и биотехнологии "Вектор"» Роспотребнадзора, п. Кольцово, Новосибирская обл., Россия*

Authors:

Volosnikova E.A., *PhD (Biology), Senior Research Associate, Head, Laboratory of Obtaining and Analizing Biosubstances, State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk Region, Russian Federation*

Shcherbakov D.N., *PhD (Biology), Senior Research Associate, State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk Region, Russian Federation*

Ermolaev V.V., *Junior Research Associate, State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk Region, Russian Federation*

Volkova N.V., *PhD (Biology), Junior Research Associate, State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk Region, Russian Federation*

Каплина О.Н. *— старший научный сотрудник ФБУН «Государственный научный центр вирусологии и биотехнологии "Вектор"» Роспотребнадзора, п. Кольцово, Новосибирская обл., Россия*

Kaplina O.N., *Senior Research Associate, State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk Region, Russian Federation*

Боргоякова М.Б. *— младший научный сотрудник ФБУН «Государственный научный центр вирусологии и биотехнологии "Вектор"» Роспотребнадзора, п. Кольцово, Новосибирская обл., Россия*

Borgoyakova M.B., *Junior Research Associate, State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk Region, Russian Federation*

Даниленко Е.Д. *— к.б.н., директор Института медицинской биотехнологии ФБУН «Государственный научный центр вирусологии и биотехнологии "Вектор"» Роспотребнадзора, п. Кольцово, Новосибирская обл., Россия*

Danilenko E.D., *PhD (Biology), Director, Institute of Medical Biotechnology, State Research Center of Virology and Biotechnology "Vector", Koltsovo, Novosibirsk Region, Russian Federation*