International Journal of Peptide Research and Therapeutics (2022) 28:156
https://doi.org/10.1007/s10989-022-10461-7

Peptide ILE-GLU-TRP (Stemokin) Potential Adjuvant Stimulating a Balanced Immune Response

Vladislav Deigin[1,2] · Dmitry Koroev[1] · Olga Volpina[1]

Accepted: 7 September 2022 / Published online: 21 October 2022

Abstract
Vaccines are widely used worldwide to prevent and protect from various infections. A variety of modern approaches to developing prophylactic and therapeutic vaccines is growing. In almost all cases, adjuvants are necessary to obtain an effective immune response.This work investigated the possibility of using the pharmaceutical peptide drug Stemokin as an adjuvant stimulating a balanced Th1/Th2 response.A study was conducted to compare the activity of Stemokin versus the approved adjuvant Alhydrogel in a murine vaccination model with the approved VAXIGRIP® vaccine.The first proof-of-concept experimental study shows that the peptide Ile-Glu-Trp has the adjuvant vaccine properties and anti-HA IgG2a enhancing response, revealing a Th1- favoring balanced Th1/Th2 immunomodulation.

Keywords Adjuvants · Peptide pharmaceutical · Balanced Th1/Th2 immune response · Hematopoiesis · Cytokines

Introduction

The pandemic situation with Covid-19 in the world has boosted the development and global use of the various prophylactic vaccines. Modern approaches to the creation of effective and selective vaccines are developing in multiple directions - from the improvement of the manufacturing methods of traditional inactivated vaccines (Sinovac) (Zhao et al. 2020; Sadoff et al. 2021), vector-bearing vaccines (J&J, Sputnik) (Logunov et al. 2020), and mRNA-based matrix (Pfizer) (Walsh, E. et al. 2020), (Moderna) (Jackson, L. A. et al. 2020) technology vaccines. On the other hand, the COVID-19 is spreading worldwide and infecting many people due to inadequate prophylactic measures. Therefore, the demand for Vaccines is overwhelming. Furthermore, the Vaccine must induce immunity quickly with the minimum amount of the antigen required. Thus, selecting an effective adjuvant becomes vital for developing an efficient Vaccine.

In the unprimed immune system, the invaded antigens are typically poor as a stimulant of the specific immune response, especially in vaccines where the immunizing antigen is an isolated individual DNA, RNA, and protein molecule or a synthetic polypeptide.

The organism's harmful action induced by the invaded antigen stimulates an immediate reaction to generate strong protection. In response to the invasion, T lymphocytes, as a significant source of protective cells, start to create cytokines. There are two main subsets of T lymphocytes, distinguished by cell surface molecules known as CD4+. A different pattern of cytokine release defines CD4 + helper cell subgroups. The Th1 subset produces a cytokine profile to induce cell-mediated immunity, and the Th2 subgroup makes a cytokine profile to induce antibody synthesis. Both subgroups act to secure and enhance a balanced immune response. In other words, the optimal scenario would seem to be that humans should produce a well-balanced Th1/Th2 response suited to fight the immune challenge. (Gupta and Gupta 2020; Kool et al. 2008).

For effective vaccination, selecting the appropriate adjuvant type for robust antibody generation and the desired antigen-specific solid immune response is essential for vaccine development. The incorporation of a conventional adjuvant along with antigens in vaccine formulation is a well-established practice in experimental immunology. Aluminum hydroxide-based suspensions are the most used adjuvants.

✉ Vladislav Deigin
vdeigin8@gmail.com

[1] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya 16/10, 117997 Moscow, Russia

[2] Immunotech developments Inc, 2395 Speakman Drive, Suite 2025, L5K 1B3 Mississauga, ON, Canada

For example, the 2% Aluminum hydroxide (Alum) wet gel suspension Alhydrogel adjuvant has been approved as an adjuvant in multiple licensed vaccines for human use in many countries worldwide (Morefield GL. et al. 2005).

The mechanism of Alhydrogel action is complex. However, depot formation at the injection site has been shown, allowing for a slow antigen release. This technology prolongs the interaction time between the antigen and the antigen-presenting cells (APCs) (Sutterwala FS, Flavell R.A, 2008). In addition, Alhydrogel is generally known to provoke a robust Th2 response, directly stimulating monocytes to produce pro-inflammatory cytokines (Kool et al. 2008). However, the use of Alum as an adjuvant has several limitations. First, the Aluminum adjuvant-containing vaccines can cause post-immunization headaches, arthralgia, and myalgia (Lindblad 2004; Lew et al. 1988).

The number of modern adjuvants approved for clinical use in humans is limited. For example, no peptide adjuvants have been registered for human use.

Some main requirements for a new generation of adjuvant activities are described in (Gupta and Gupta 2020).

Therefore, a novel adjuvant needs to be developed to produce vaccines that cannot currently be adjuvanted by Alum-based vaccination strategies (Geerligs et al. 1989).

A new promising direction in developing a new generation of adjuvant is the use of immune- and hemopoietic peptides. A more intensive study of peptide preparations involved in regulating the immune response and hematopoiesis will pave a new prospect for its use as effective and safe adjuvants.

Isolation of the biologically active peptide Glu-Trp from the thymus gland (Morozov V.G. Khavinson, V.K, 1997) was followed by a series of structure-functional studies (SAR) of related linear and cyclic peptides (Deigin, V.I. et al. 1999, Deigin, V.I. et al. 2007, Deigin, V.I. et al., 2000, Semina, O.V. et al. 1997, Vinogradova, Yu et al. 1999).

As a result, an original tripeptide Ile-Glu-Trp, which showed hemostimulating activity, stimulating the proliferation of hematopoietic stem cells damaged by radiation or cytostatic action, has been discovered.

A study of the pharmacokinetics of H^3-(Ile-Glu-Trp) showed that the intramuscularly administered peptide predominantly accumulates in bone marrow (45%) (Semina, O.V. et al. 1997). Therefore, a medicinal product based on this peptide is registered in Russia as an immune-and hemostimulatory agent under the trade name Stemokin® (Deigin, V.I. et al. 2007).

Unlike the known peptide immunomodulators Zadaxsen (E.Garachi. 2007), Immunox (Goldstein et al. 1979), Thymogen (Deigin, V.I. et al. 1999), Likopid (Meshcheryakova et al. 2007), Stemokin acts on earlier stages of hematopoiesis, including CD34 cells, at the same level as other hemoregulatory Granulocyte- and Granulocyte-macrophage colony-stimulating factors (CSF, G-CSF, and GM-CSF) (Semenets, T.N. et al. 2000).

The experimental and clinical information available for Stemokin (Deigin, V.I. et al. 1999, (Vladislav Deigin, et.al., 2020, Deigin, V.I. et al. 2007, Deigin V. 2012, Deigin V.I et al 2016, Deigin, V.I. et al., 2000, Semina, O.V. et al. 1997, Vinogradova, Yu et al. 1999) is applied to undertake the *first pilot proof of concept* experiment directed to use Stemogen as a "modern adjuvant" (Gupta and Gupta 2020).

This study aims to compare the potential adjuvant activity of Stemokin versus the approved adjuvant Alhydrogel in a murine vaccination model with the approved VAXIGRIP® Vaccine (Hannoun 2013).

Vaxigrip vaccines (Vaxigrip®, IIV3, and VaxigripTetra™ IIV4) from Sanofi Pasteur are registered flu-preventing vaccines (Hannoun 2013); this Vaccine contains Alhydrogel as an adjuvant. In addition, in the 1980s, the trivalent Vaccine Vaxigrip™ – Inactivated Influenza Vaccine, Trivalent Types A and B, was developed (Viviane Gresset-Bourgeois et al. 2017). Vaxigrip influenza vaccines provide an opportunity to reduce further the morbidity and mortality associated with annual influenza epidemics.

Materials & Methods

Animals

The procedures performed in this study followed the Guide for the Care and Use of.Laboratory Animals published by the National Institutes of Health and with the "Regulations. for Studies with Experimental Animals" (Decree of the Russian Ministry of Health from.Aug 12, 1997, No. 755). The Institutional Ethics approved the protocol.Committee of the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry (Protocol No.161/2015). All efforts were taken to minimize suffering.Balb/c female mice are aged 9–11 weeks (Harlan Laboratories, Canada). The initial weight range for use in the study was 19–21 g. The mice were housed in micro isolator cages. Mice were s.c. injected with 100 µl/mouse. All groups (10 mice in a group) received one administration on Day 1 and Day 28.

Test Article

Stemokin Sodium Chemical Name: L-Isoleucyl-L-Glutamyl-L-Tryptophan Monosodium salt.

Purity: 99.8% (LCMS UPLC Waters Acuity System). (Immunotech Developments Inc. Canada).

Table 1 The distribution of the treatment groups

Group	Treatment on each dosing day*
1	Vaxigrip alone **(V)**
2	Vaxigrip + Stemokin (1 µg) **(V + S1 µg)**
3	Vaxigrip + Stemokin (25 µg) **(V + S25 µg)**
4	Vaxigrip + Stemokin (100 µg) **(V + S100 µg)**
5	Vaxigrip + Alhydrogel (10 µg) **(V + Alum)**

*In all groups Vaxigrip contains 0,33 µg HA(H1N1) and 0,33 µg HA(H3N2)

Influenza Vaccine

Vaxigrip is an inactivated influenza vaccine trivalent Types A and B (split virion), manufactured and distributed by Sanofi Pasteur Limited, Toronto, Canada. It is prepared from a virus grown in the allantoic cavity of embryonated eggs. The virus is purified by zonal centrifugation on a sucrose gradient, dissolved in the surfactant octoxinol 9 (Triton® X100), inactivated in formaldehyde, and then diluted in phosphate-buffered saline. For the experiment, each 0.5 mL dose of Vaxigrip® contains 15 µg HA A/B (H1N1)-like strain and 15 µg HA A/B (H3N2)-like strain. Other Ingredients are ≤ 30 µg formaldehyde, up to 0.5 mL sodium phosphate-buffered, isotonic sodium chloride solution, 2 µg thimerosal as a preservative, and the surfactant Triton® X100. There is no adjuvant presented.

Adjuvant: Aluminum Hydroxide

Name: "Alhydrogel"; (Accurate Scientific, USA).

Scheme of the Experiment

Formulation of the Test Articles

Stock Solutions (20 mg/mL and 0.5 mg/mL) of Stemokin were prepared in sterile, non-pyrogenic saline. The solutions were filtered through a sterile, non-pyrogenic PVDF membrane filter with porosity NMT 0.2 µm. 222 µL of Vaxigrip suspension was diluted with the saline to make 2000 µL solution (group 1) or diluted with to make 2000 µL of solution after adding the calculated amount of stock solutions of Stemokin or Alhydrogel (groups 2–5).

Mice Treatment

Ten mice each were randomized into treatment groups. A 100 µL of the appropriate Formulation for the allocated group was injected as a bolus subcutaneously on Day 1 and then again on Day 28.

Blood Collection

Blood was collected on days 14, 28, and 42 from the saphenous vein without anticoagulants, and serum was separated and stored at -70^0 C until use in serological assays.

IgG Isotype ELISAs (IgG)

Briefly, ELISA plates were directly coated with the Vaxigrip. Next, dishes were washed with PBS containing 0.05% Tween 20 (T-PBS) and blocked with 1% bovine serum albumin (BSA) for one h at 37 °C. Next, antigen-coated plates were washed with T-PBS and incubated with 1:1.000-diluted individual mice serum samples overnight at 4 °C. After washing with T-PBS, plates were incubated with goat anti-mice IgG1 or IgG2a horseradish peroxidase conjugates (Rockland Immunochemical) in a 1:10,000 dilution for 2 h at 37 °C. The reaction was developed by o-phenylenediamine for 30 min and read the optical density at 450 nm. Influenza-specific ELISA titers were extrapolated by linear regression from a standard curve and expressed as ug/ml. Averages were presented for both IgG isotypes.

Hemagglutinin Inhibition (HI) test

Mouse serum samples were treated with receptor destroying enzyme (RDE) at 37 °C overnight. Fresh turkey red blood cells (TRBC) were washed and diluted in PBS to a concentration of 0.5% (vol/vol). The mice sera were diluted in PBS in 96-well V-bottom cell culture plates. The serially diluted pool sera from each mice group were incubated with the H3N2 Influenza strain for 15 min. Fifty microliters of 0.5% TRBC were then added, and the plates were incubated at room temperature for 30 min. The hemagglutination inhibition (**HI**) titer was the reciprocal of the highest serum dilution to prevent agglutination completely. The $\log_2$ transformed geometric mean titers for each group are presented.

Statistical Analysis

The student's *t*-test has been used to compare data differences in antibody concentrations and titers for each mice group vs. V and V + Alum groups. The data presented as $\bar{x}$ ± SEM.

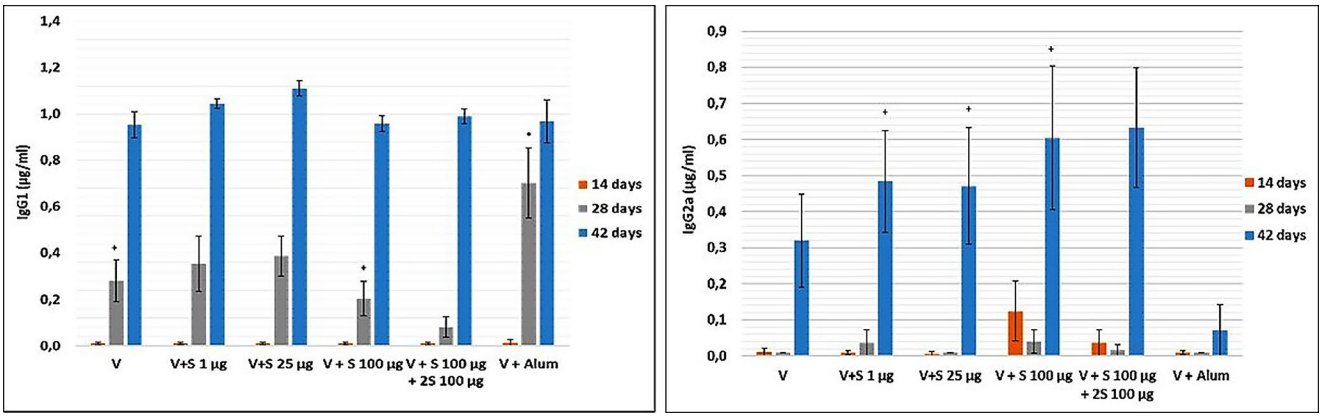

Fig. 1 Antibody response to Vaxigrip antigen after immunization
* $p < 0.05$ vs. V group, $^{+}$ $p < 0.05$ vs. (V + Alum) group

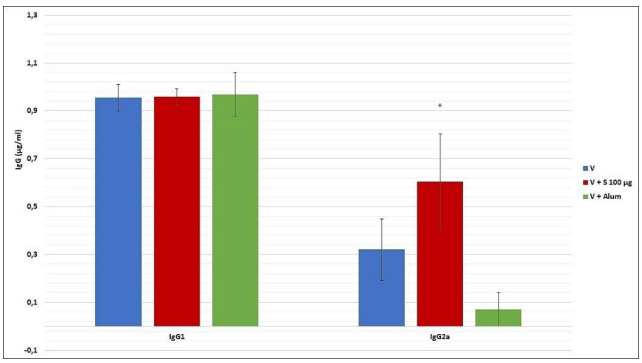

Fig. 2 The comparison of IgG1 and IgG2a antibodies to Vaxigrip at day 42
$^{+}$ $p < 0.05$ vs. (V + Alum) group

Results

Pilot experiments to evaluate the adjuvant potential of Stemokin have been performed in an experimental murine model with Influenza Virus Vaccine (Vaxigrip™) as an immunogen (Hannoun 2013). The approved Alhydrogel adjuvant has been used as a comparison preparation (Kool et al. 2008).

Mice were immunized in three variants with Vaxigrip - without adjuvants, Alhydrogel adjuvant, and three doses (1, 25, and 100µ) of Stemokin as an adjuvant. (Fig. 1).

This experiment estimates anti-influenza antibodies of IgG1 and IgG2a isotypes and Stemokin adjuvanticity compared to Alhydrogel in inducing specific anti-hemagglutinin antibodies after Vaccine immunization.

At a different time, the data shows that Stemokin treated groups boosted the IgG2a antibody response two weeks earlier than the Aluminum (V + Alum) group.

As shown in Fig. 1, on day 28 after the first immunization, the (V + Alum) mice group stimulated significantly higher IgG1 levels than the V and (V + S100 µg) groups. At the same time, it was no difference in the concentration of IgG1 on day 42 for all groups. The different response was with IgG2a amounts; on day 42, the concentration of the IgG2a in all groups received (V + S) was higher than for the (V + Alum) group. Thus, the mice group received (V + Alum) demonstrated an early IgG1 response, while the induction of IgG2a antibodies can only be stimulated by animals that received V with Stemokin at various doses.

The Alhydrogel treated group showed early stimulation of a Th2 response, consistent with promoting humoral immunity in preference to a cellular response. Successively high Stemokin dose in the vaccine composition produced progressively higher Vaxigrip + Alhydrogel IgG2a response.

This pilot experiment shows that Stemokin has the potency of a vaccine adjuvant favoring a balanced Th1/Th2 response represented as IgG1 and IgG2a in the combined Vaccine/Stemokin group compared to the Vaccine + Alhydrogel group (Fig. 2).

To evaluate the Hemagglutinin Inhibition (**HI**) ability by the Stemokin (V + S100) and Alhydrogel (V + Alum) groups, we tested mouse serum samples against the H3N2 Influenza strain. The **HI** titer was the reciprocal of the highest serum dilution to prevent agglutination completely. The $\log_2$ transformed geometric mean titers for each group are presented in Fig. 3. All studied groups on day 42 showed high **HI** activity, and there was no significant difference between the sera of all studied groups; however, the high dose of the Stemokin adjuvant group (V + S 100 µg) on Day 42 tends to inhibit the **HI** titers compared to Vaccine + Alhydrogel (V + Alum) group.

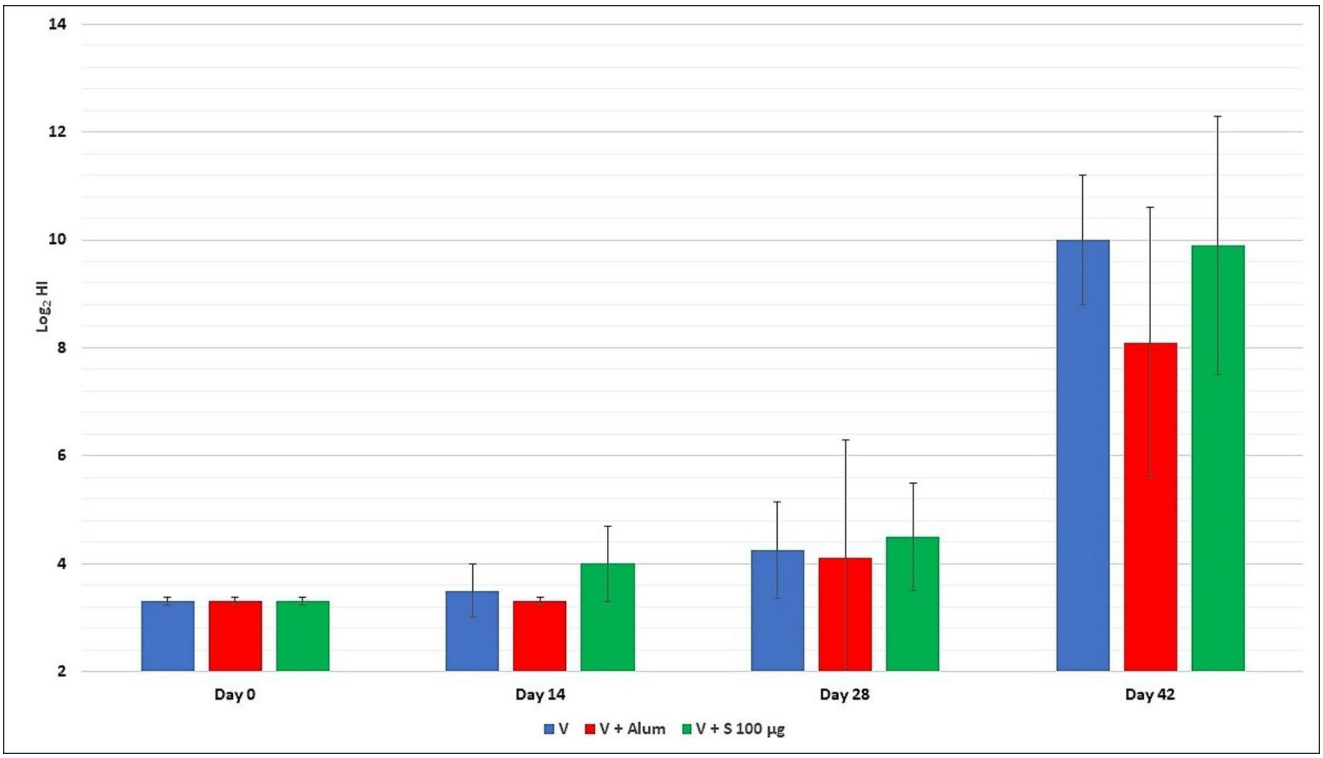

Fig. 3 Hemagglutinin inhibition (**HI**) of mice sera after immunization with Vaxigrip (V), Stemokin (V + S100 µg), and Alhydrogel (V + Alum) group

Discussion

The critical factor in vaccine design is ensuring the Vaccine's efficacy while reducing the potential risks associated with it.

The immune response induced by antigenic or genetic immunization can thus generally be distinguished by these two subtypes: Th1/Th2. Successful vaccines for most pathogens will require enhanced immune responses, including Th1-cellular-mediated immunity and robust Th2-humoral response. It is necessary to vaccinate twice or three times to obtain an intense and long-lasting immune response. An essential mechanism in the immune regulation involves homeostasis between the Th1 and Th2 activity of CD4 + T helper cells expressing different cytokine patterns. (Gupta and Gupta 2020; Kool et al. 2008).

Adjuvants may exhibit their immunostimulatory effects via various mechanisms: providing antigen depot; activation of innate immunity through pathogen recognition receptors engagement; co-stimulation of immune cells; and immunomodulation, e.g., maturation of particularly dendritic cells (Joffre O.P. et al. 2012).

Various experimental studies are performed with peptides as potential adjuvants.

In 1975, the active subunit of bacterial cell walls in Freund's complete adjuvant was identified and synthesized (Audibert et al. 1982; Ellouz et al. 1974, Kotani, S. et al. 1975). Peptidoglycan MDP and most analogs enhance humoral antibody production, demonstrated with classical laboratory antigens and conventional vaccines against viral, bacterial, or parasitic pathogens (Siddiqui, W.A. et al. 1978). In addition, it is reported that MDP (Girardin et al. 2003) and their disaccharide derivative GMDP (Meshcheryakova et al. 2007) confer their adjuvanticity by activating the NF-κB pathway through the NOD2 receptor.

Another application of peptide adjuvants is peptide amphiphile micelles (PAMs). These compounds have been studied as vaccine carriers, inducing strong antibody response (Barrett J.C. et al. 2017, Trent et al. 2015).

The literature (Zhang R 2018) shows that a single immune agonist is not always sufficient to elicit an efficacious immune response. Usually, Aluminum hydroxide-based adjuvant vaccines are directed to prime the Th2 immune response rather than a Th1-biased response.

Composite adjuvants containing Aluminum and immunomodulatory peptides could be used to enhance the immune response (Chang T.Z. et al. 2017, Deng et al. 2018) synergistically. According to the specific immune response generated by different adjuvants, several composite adjuvant systems might find clinical applications (Wang et al. 2017, Sutherland D.R. et al. 1996).

Most composite adjuvants are heterogeneous emulsions, leading to local and systemic adverse reactions (Lindblad 2004; Lew et al. 1988).

In addition to a more balanced immune response, Stemokin solution as an adjuvant does not cause local complications compared to most composite adjuvants. Because Stemokin acts on earlier stages of hematopoiesis, including CD34 cells, stimulating the proliferation of hematopoietic cells at the same level as other hemoregulatory factors we have compared the Stemokin effect with (G-CSF and GM-CSF) (Francisco-Cruz et al. 2014; Souza et al. 1986) on hematopoietic cells. After treatment by Stemokin the total bone marrow cells amount was restored faster than the GM-CSF group. Furthermore, the cellularity volume in mice treated with Stemokin completely recovered two times more intensive than in the group treated with GM-CSF (Semenets, T.N. et al. 2000).

Given the close interaction of the hematopoiesis and immune system (Na Li et al. 2017), we have evaluated the Stemokin adjuvanticity compared to Alhydrogel.

The segregation of IgG1 and IgG2a immunoglobulin isotypes as markers for Th2 and Th1 lymphocytes, respectively, was investigated.

For this study, the influenza vaccine Vaxigrip was used to immunize mice in three variants - without adjuvants, Alhydrogel adjuvant, and three Stemokin doses.

After the first immunization, the (V + Alum) treated group on Day 28 elicited significantly higher IgG1 levels than the V and (V + S100 µg) groups. However, at the further immunization, the difference in the concentration of IgG1 on day 42 for all groups was insignificant.

At the same time, the concentration of the IgG2a in all groups received (V + S) on day 42 was higher than for the (V + Alum) group. The mice group received (V + Alum) demonstrated an early IgG1 response, while the induction of IgG2a antibodies can only be stimulated by animals that received (V + S1 µg), (V + S 25 µg), and (V + S100 µg) doses.

These experimental results showed the early stimulation of Th2 response by the Alhydrogel treated group consistent with promoting humoral immunity in preference to a cellular one. At the same time, successively high Stemokin dose in the vaccine composition produced progressively higher IgG2a response comparable to Vaxigrip + Alhydrogel IgG1 response.

Using a Stemokin in the composition makes it possible to generate a substantial humoral and T- cell response, balancing Th1/Th2 better than generally obtained by standard Alhydrogel (V + Alum) vaccine adjuvant use.

Conclusion

This first proof-of-concept pilot experimental study provides evidence that a pharmaceutical preparation Stemokin has the potency of a vaccine adjuvant favoring a balanced Th1/Th2 response.

Following Stemokin treatment, anti-HA IgG2a response was enhanced, revealing a Th1- favoring balanced Th1/Th2 immunomodulation. In other words, Stemokin promotes a more rapid protective response.

The optimized Stemokin concentration in the specific immunogen/adjuvant composition can generate a higher IgG2a (Fig. 1) response and balance the Th1/Th2 ratio more efficiently than a traditional vaccine adjuvant.

More detailed studies of Stemokin and its analogs as adjuvants are planned with various types of modern vaccines.

Authors Contributions Vladislav Deigin: Peptide synthesis, wrote the main manuscript text.Dmitry Koroev: prepared all figures and statistical analysis.Olga Volpnia: immunological testing, wrote the main manuscript text.

Declarations

Competing Interests The authors declare no competing interests.

References

Audibert F, Przewlocki G, Lefrancier P, Choay J, Chedid L (1982) Reinforcement de l'activite d'un vaccin contre l'hepatite virale de type B par association avec Ie murabutide. C.R. Acad, Sc. Paris 295: 611–614

Barrett JC, Ulery BD, Trent A, Liang S, David NA, Tirrell MV (2017) Modular peptide Amphiphile micelles improving an antibody-mediated immune response to group A Streptococcus. ACS Biomater Sci Eng 3(2):144–152

Chang TZ, Stadmiller S, Staskevicius E, Champion JA (2017) Effects of ovalbumin protein nanoparticle vaccine size and coating on dendritic cell processing. Biomater Sci 5(2):223–233

Deigin VI, Poverenny AM, Semina OV, Semenets TN (1999) Reciprocal effect of optical isomerism of EW-dipeptides on immune response. Immunol Lett 67:41–46

Deigin VI, Poverenny AM, Semina OV, Semenets TN (2000) Stimulation and suppression of the immune response and hemopoiesis by novel natural and synthetic peptide. Peptides for the New Millennium. American Peptide Symposia; Springer, Berlin/Heidelberg, Germany, p 741

Deigin V, Ksenofontova O, Khrushchev A, Yatskin O, Goryacheva A, Ivanov V, – (2016) Chemical platform for the preparation of synthetic orally active peptidomimetics with hemoregulating activity. J ChemMedChem Vol 11:1974–1977

Deigin V (2012) Novel branched diketopiperazines as a universal scaffold for orally active small peptide derivatives, Peptides 2012. Proceedings of the 32 European peptide symposia. Journal of Peptide Science. - P. 98–99. IF: 1,951

Vladislav Deigin O, Ksenofontova O, Yatskin A, Goryacheva A, Ignatova V (2020) Ivanov Novel platform for the preparation of synthetic orally active peptidomimetics with hemoregulating activity.

II. Hemosuppressor activity of 2,5-diketopiperazine-based cyclopeptides//. International Immunopharmacology 81 106185
Deigin VI, Semenets TN, Zamulaeva IA, Maliutina YV, Selivanova EI, Saenko AS, Semina OV (2007) The effects of the EW dipeptide optical and chemical isomers on the CFU-S population in intact and irradiated mice. Int Immunopharmacol 7:375–382
Deng L, Mohan T, Chang TZ, Gonzalez GX, Wang Y, Kwon Y-M et al (2018) Double-layered protein nanoparticles induce broad protection against divergent influenza viruses. Nat Commun 9(1):359
Ellouz F, Adam A, Ciorbaru R, Lederer E (1974) Minimal structural requirements for adjuvant activity of bacterial peptidoglycan derivatives. Biochem Biophys Res Commun 59:1317–1325
Francisco-Cruz A, Aguilar-Santelises M, Ramos-Espinosa O, Mata-Espinosa D, Marquina-Castillo B, Barrios-Payan J, Hernandez-Pando R (2014) "Granulocyte-macrophage colony-stimulating factor: not just another hematopoietic growth factor". Med Oncol 31(1):774. doi:https://doi.org/10.1007/s12032-013-0774-6
E.Garachi (2007) Thymosin alpha 1: a historical overview. Ann N Y Acad Sci 1112:14–20
Geerligs HJ, Weijer WJ, Welling GW, Welling-Wester S (1989) The influence of different adjuvants on the immune response to a synthetic peptide comprising amino acid residues 9–21 of herpes simplex virus type 1 glycoprotein D. J Immunol Methods 124:95–102 PMID:2553820. https://doi.org/10.1016/0022-1759(89)90190-7
Girardin S et al (2003) Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 278(11):8869–8872
Viviane Gresset-Bourgeois PS, Leventhal, Stéphanie Pepin R, Hollingsworth, Samson (2017) Quadrivalent inactivated influenza vaccine (VaxigripTetra™), Expert Review of Vaccines, DOI:https://doi.org/10.1080/14760584.2018.1407650
Goldstein G, Scheid M, Boyce EA, Schlesinger DH, Wauwe JVan (1979) A synthetic pentapeptide with biological activity characteristic of thymic hormone thymopoietin. Science 204:1309–1310
Gupta T, Gupta SK (2020) Potential adjuvants for developing a SARS-CoV-2 vaccine based on experimental results from similar coronaviruses, International Immunopharmacology (2020), DOI: https://doi.org/10.1016/j.intimp.2020.10671
Hannoun C (2013) The evolving history of influenza viruses and influenza vaccines. Expert Rev Vaccines 12:1085–1094
Jackson LA et al (2020) An mRNA vaccine against SARS-CoV-2—Preliminary Report. N Engl J Med. https://doi.org/10.1056/NEJMoa2022483(2020)555.]
Joffre OP, Segura E, Savina A, Amigorena S (2012) Cross- presentation by dendritic cells. Nat Rev Immunol. 12:557 – 69; PMID:22790179 https://doi.org/10.1038/nri3254
Kool M, Soullié T, van Nimwegen M, Willart MAM, Muskens F, Jung S, Hoogsteden HC, Hammad H, Lambrecht BN (2008) Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. J Exp Med 2008; 205:869 – 82; PMID:18362170; https://doi.org/10.1084/jem.20071087
Kotani S, Watanabe Y, Shimono T, Narita T, Kato K, Stewart-Tul DES 1, Kinoshita F, Yokogawa K, Kawata S, Shiba T, Kusumoto S, Tarumi Y (197) The immunoadjuvant activities of cell wall1s, their water-soluble fractions, and peptidoglycan subunits were prepared from various gram-positive bacteria and synthetic N-acetylmuramyl peptides. Z. Immunitatsforsch. 1498: 302–319
Lew AM, Anders RF, Edwards SJ, Langford CJ (1988) Comparison of antibody avidity and titer elicited by peptide as a protein conjugate or as expressed in vaccinia. Immunology 65:311–314 PMID:3056855
Lindblad EB (2004) Aluminum adjuvants in retrospect and prospect. Vaccine 22:3658–3668 vaccine.2004.03.032. http://dx.doi.org/10.1016/j.vaccine.2004.03.032
Logunov DY, Dolzhikova IV, Zubkova OV (2020) Safety and immunogenicity of a rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 Vaccine in two formulations: two open, non-randomized phase 1/2 studies from Russia, Lancet; 396: 887–97
Meshcheryakova E, Makarov E, Philpott D, Andronova T, Ivanov V (2007) Evidence for a correlation between the intensities of adjuvant effects and NOD2 activation by monomeric, dimeric, and lipophilic derivatives of N-acetylglucosaminyl-N- acetylmuramyl peptides. Vaccine 25:4515–4520. https://doi.org/10.1016/j.vaccine.2007.04.006
Morefield GL et al (2005) Role of aluminum-containing adjuvants in antigen internalization by dendritic cells in vitro. Vaccine 23(13):1588–1595
Morozov VG, Khavinson VK (1997) Natural and synthetic thymic peptides as therapeutics for immune dysfunction. Int J Immunopharmacol 19:501–505
Li N, Jinlian Hua (2017). Interactions between mesenchymal stem cells and the immune system. Cell. Mol. Life Sci. 2017DOI https://doi.org/10.1007/s00018-017-2473-5
Sadoff J, Gray G, Vandebosch A et al (2021) Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19. N Engl J Med 384:2187–2201
Semenets TN, Semina OV, Vinogradova YE, Deigin VI, Poverenny AM (2000) Use of synthetic immunomodulating peptides to restore hematopoiesis in mice after cytostatic cytosine-arabinoside (Ara-C). *Immunology* 2000, *6*, 20–22
Semina OV, Semenets TN, Semina OV, Semenets TN, Deigin VI et al (1997) Thymogen stimulation (Glu-Trp dipeptide) to restore hematopoiesis in irradiated and cytostatic-exposed mice // Immunology.N1- p.33–35
Siddiqui WA, Taylor DW, Kan SC, Kramer K, Richmond-Crum SM, Kotani S, Shiba T, Kusumoto S (1978) Vaccination of experimental monkeys against Plasmodium falciparum: a possible safe adjuvant. Science 201:1237–1239
Souza LM, Boone TC, Gabrilove J et al (1986) "Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. Sci Apr 4(4746):61–65
Sutherland DR et al (1996) The ISHAGE guidelines for CD34 + cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering J Hematother V. 5. – № 3. – P.213–226
Sutterwala FS, Flavell RA (2008) Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminum adjuvants. Nature 453:1122–1126 PMID:18496530. http://dx.doi.org/10.1038
Trent A, Ulery BD, Black MJ, Barrett JC, Liang S, Kostenko Y et al (2015) Peptide amphiphile micelles self-adjuvant group A streptococcal vaccination. AAPS J 17(2):380–388
Vinogradova, Yu E, Deigin VI, Korotkov AM, Semina OV, Sements TN, Poverenny AM (1999) The use of Thymogen for the treatment of patients with diseases of the blood system. 1. The effect of Thymogen on the granulocytic lineage of hematopoiesis in patients with depression of hematopoiesis. Russ J Oncol 2:45–48
Walsh EE et al (2020) RNA-based COVID-19 vaccine BNT162b2 selected for a Pivotal Efficacy Study, medRxiv. https://doi.org/10.1101/2020.08.17.20176651
Wang L, Chang TZ, He Y, Kim JR, Wang S, Mohan T et al (2017) Coated protein nanoclusters from influenza H7N9 HA are highly immunogenic and induce robust protective immunity. Nanomedicine 13(1):253–262
Zhang R, Ulery BD (2018) Synthetic vaccine characterization and design. J Bionanosci 12(1):1–11
Zhao J, Zhao S, Ou J et al (2020) "COVID-19: Coronavirus Vaccine Development Updates". Front Immunol V 11 1–19. DOI: https://doi.org/10.3389/fimmu.2020.602256