MATKA-APURAHARAPORTTI

Infektiotautien tutkimusyhdistyksen matka-apuraha 2023

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Virologian ja rokoteimmunologian tutkimusryhmä
Lääketieteen ja terveysteknologian tiedekunta
Tampereen yliopisto

Kongressimatka IMMUNOLOGY2023™ -kongressiin

IMMUNOLOGY2023™ on American Association of Immunologists (AAI) -järjestön vuosittain järjestämä kongressi, joka tänä vuonna pidettiin Washington DC:ssä 11.-15. toukokuuta. Tämä kongressi on yksi maailman suurimmista immunologian tieteenalaan keskittyvistä kongresseista ja tänäkin vuonna kongressiin submittoituja abstrakteja oli yli 2000. Viisipäiväisen kongressin aikana sukelsimme syvälle immunologian maailmaan ja opimme paljon rokotekehityksestä sekä rokotteiden aikaansaamien immuunivasteiden analysoinnista. Kongressipäivät koostuivat pääosin eri aihealueiden symposiumeista, jotka sisälsivät 15-30 minuutin kestoisia esityksiä. Ensimmäisenä päivänä osallistuimme Vaccination and Vaccine-Induced Responses against Pathogens at Target Sites -symposiumiin. Toisena päivänä osallistuimme useampaan symposiumiin: Approaches to Improve Vaccination and Immunotherapy against Pathogens, Immunological Approaches to (Re)emerging and Global Zoonotic Threats & Immune Responses in the Respiratory Tract. Lisäksi toisena päivänä alkoivat posteriesitykset. Kolmantena päivänä oli Saanan posteriesityksen vuoro ja lisäksi osallistuimme kahteen symposiumiin Molecular Mechanisms of Cytokine Function ja Vaccination against Pathogens at different Stages of Life and Disease. Neljäntenä päivänä osallistuimme Immunity to SARS-CoV-2-symposiumiin sekä AAI:n puheenjohtajan järjestämään symposiumiin, jonne hän oli kutsunut alansa arvostetuimpia tutkijoita luennoimaan. Luentojen aiheina olivat mm. koronarokotteiden kehitys, immuunivasteet SARS-CoV-2 -infektiossa sekä ihmisen immuunijärjestelmän kehitys. Lisäksi neljäntenä päivänä oli Stinan posteriesityksen vuoro. Viimeisenä päivänä osallistuimme vielä isoon symposiumiin, jonka aiheena oli Immunity to Emerging Pathogens: COVID-19 and Beyond.

Kaiken kaikkiaan kongressi oli erittäin opettavainen. Tämä oli ensimmäinen iso maailmanlaajuinen kongressi, johon osallistuimme. Johtuen kongressin sijainnista Washington DC:ssä kongressissa oli useita luentoja ja posteriesityksiä Yhdysvaltojen terveysviraston (National Institutes of Health) tutkimusryhmiltä. Oli mielenkiintoista kuulla heidän tutkimuksistaan. Oma tutkimusryhmämme Tampereen yliopistolla on vielä melko pieni, joten oli myös mielenkiintoista nähdä suurien yhdysvaltalaisyliopistojen tutkimusryhmien tutkimuksia ja kuinka paljon isommat resurssit heillä on tutkimuksen tekoon.

Isot kiitokset ITY:lle matka-apurahojen myöntämisestä! Myönnetyt matka-apurahat auttoivat kustantamaan kongressin rekisteröitymismaksut, abstraktien submittoimismaksut, posterien painatukset sekä osan majoituskustannuksista. Alla liitteenä meidän posterimme.

Hexavalent coronavirus vaccine elicits both humoral and cellular immune responses in mice

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Abstract

Coronaviruses (CoVs) have caused three deadly human outbreaks over the last 20 years, the latest causing the COVID-19 pandemic which has now lasted over three years. The animal reservoir of coronaviruses is extensive, and history has shown that coronaviruses are able to crossover from animals to humans. Thus, there is a clear need for a universal coronavirus vaccine. In this study, we produced S1 subunits of spike proteins of six different human coronaviruses fused to the antibody Fc fragment in HEK293 cells. In addition, we produced another coronavirus protein (CP) in High Five™ insect cells. The vaccine compositions included either the six S1-Fc or the six S1-Fc together with CP, and mice were immunized either intranasally or with a combination method utilizing both subcutaneous and intranasal injections. Our results showed that both vaccine compositions elicited both humoral and cellular immune responses in mice. Antigen-specific IgG levels were higher in mice immunized solely intranasally but using a combination of different immunization routes seemed to boost cellular immune responses. As a conclusion, all the vaccine compositions were highly immunogenic and generated both antibody and T cell responses in mice. Further studies are needed to optimize the immunization scheme. Additionally in the future, in order to better characterize the T cell responses generated by intranasal immunization, the nasal associated lymphoid tissue and cervical lymph nodes from the mice will be collected and analyzed.

Expression and purification of antigens

S1 domains of spike proteins (amino acid residues 16–541) of MERS-CoV, HCoV-OC43, HCoV-229E, HCoV-HKU1, HCoV-NL63, and SARS-CoV-2 (omicron BA.1) were expressed as fusion proteins with mouse IgG2a Fc fragment (amino acid residues 238–469) and 8xhistidine tag (S1-mFc-8xhis) in human embryonic kidney (HEK293F) cells. The S1-mFc-8xhis proteins were purified utilizing Ni-NTA columns, eluted with increasing imidazole concentrations, and dialyzed against phosphate-buffered saline (PBS). For ELISA assays, the same S1 domains were expressed with histidine tags but without the mouse IgG2a Fc fragments (S1-8xhis) and produced and purified similarly to the S1-mFc-8xhis fusion proteins.

Another coronavirus protein of Wuhan variant of SARS-CoV-2 (CP) was expressed in Trichoplusia ni (High Five™) insect cells utilizing the baculovirus expression system. This protein was purified with tangential flow filtration and ion exchange chromatography and dialyzed against PBS. The same construct was used for immunizations and immunoassays.

Immunizations

Female BALB/cJRj, 6 wk old, were purchased from Janvier Labs (Le Genest-Saint-Isle, France) and were maintained in our laboratory under a specific pathogen-free condition. Mice were grouped in four groups: Group 1 was given PBS, group 2 hexavalent vaccine consisting of 1µg of each S1-mFc-8xhis, group 3 hexavalent vaccine with 1µg of CP and group 4 the same vaccine as group 3. All vaccines were adjuvanted with branched polyethyleneimine (BPEI) and all the groups were immunized three times at a 4-week interval. Groups 1, 2, and 3 were immunized solely through the intranasal route, however, group 4 got their first dose subcutaneously and the rest intranasally. Blood samples were collected at wk 0, 4, and 8. The mice were terminated at wk 12, and whole blood and spleens were collected.

Immunoassays

Total IgG levels were measured from serum samples collected at weeks 4, 8 and 12 with enzyme-linked immunosorbent assay (ELISA). S1-8xhis proteins were used for coating the wells instead of S1-mFc-8xhis fusion proteins which were used for immunizations due to the anti-mouse IgG HRP conjugated secondary antibody recognizing the mouse IgG2a Fc fragment.

Simultaneous secretion of IFN-γ, IL-2, and TNF-α was analyzed with Mouse IFN-γ/IL-2/TNF-α FluoroSpotPLUS kit (Mabtech, Sweden) using cryopreserved splenocytes. The assay was set up in duplicate under sterile conditions and the splenocytes of individual mice were tested separately according to the manufacturer's instructions. In this assay, S1-mFc-8xhis fusion proteins were used as stimulants.

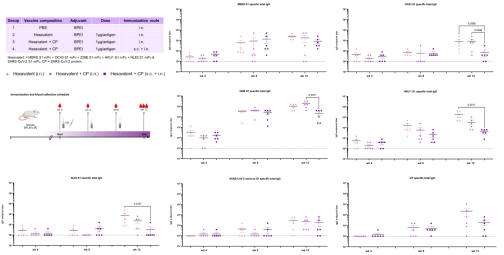

Fig. 1 Immunization scheme and antigen specific total IgG antibody levels. Undetectable antibody levels were denoted with the titer 100 (dotted line).

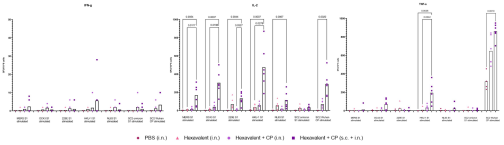

Fig. 2 Antigen stimulation specific cytokine levels

Results and conclusions

Principally, our ELISA data showed antigen specific IgG levels increasing as the number of immunizations increased (Fig. 1). After three immunizations, group 2 had significantly higher levels of OC43, HKU1, and NL63 specific IgG antibodies than group 4. In addition, group 3 had significantly higher levels of OC43 and 229E specific IgG antibodies compared to group 4. 229E S1-mFc-8xhis seemed to elicit higher IgG antibody levels already at wk 8 and be immunodominant compared to the other fusion proteins.

When the splenocytes of group 4 were stimulated with S1-mFc of MERS, OC43, 229E, HKU1, or NL63 or with CP, the numbers of IL-2 secreting splenocytes were significantly higher than those of group 1(Fig. 2). In addition, when stimulating with S1-mFc of MERS, OC43, or HKU1 the numbers of IL-2 secreting splenocytes of group 4 were significantly higher than those of group 2. The numbers of TNF-α secreting splenocytes were significantly higher with group 4 than those of group 1 when the splenocytes were stimulated with S1-mFc of HKU1, or with CP.

Our results showed that both vaccine compositions (hexavalent and hexavalent + CP) elicited both humoral and cellular immune responses in mice. Antigen-specific IgG levels were higher in mice immunized solely intranasally but using a combination of different immunization routes seemed to boost cellular immune responses. As a conclusion, all the vaccine compositions were highly immunogenic and generated both antibody and T cell responses in mice. Further studies are needed to optimize the immunization scheme and dosage of the antigens.

Funding

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Characterization of immune response towards modified Coxsackie B virus-like particle vaccine in a murine model

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ABSTRACT

Coxsackievirus Bs (CVBs) are common human RNA viruses that belong to the enterovirus genus of *Picornaviridae*. Most commonly, infections with these viruses manifest with mild flu-like symptoms. However, CVBs are also known to cause severe diseases, including aseptic meningitis, myocarditis, and chronic dilated cardiomyopathy (DCM), encephalitis, pancreatitis, and hand-foot-and-mouth disease. In this study, we aimed for a versatile characterization of a modified CVB virus-like particle (VLP) vaccine in a murine model. The modified CVB-VLP was produced using baculovirus-insect cell production platform. Purified and characterized vaccine was administered to mice via s.c. or i.m. routes, either with or without an adjuvant. Immunological characterization of vaccine response included both humoral and cellular characterization. Antigen-specific IgG antibodies in the sera were measured to determine the humoral immune response. To characterize the cellular immunity, activation markers of the lymphocytes were identified using flow cytometry. Additionally, the cytokine secretion of the stimulated splenocytes was determined with FluoroSpot assay. The modified CVB-VLP induced high levels of antigen-specific IgG antibodies in the sera of vaccinated mice. Further, the in vitro stimulation of vaccinated mice splenocytes with infective CVB induced activation of mainly CD4+ T-cells, whereas in the adjuvanted vaccine group, a significant production of IFN-γ and IL-2 was induced. These results indicate that the virus-like particle combined with an adjuvant is a promising vaccine candidate. In addition, our ongoing challenge study with infective CVB will shed us more insight on the protective role of this vaccine.

STEP 1. VACCINE PREPARATION

The modified CVB-VLPs were produced in insect cells and purified through Tangential Flow Filtration and Multistep-Ion Exchange Chromatography. The vaccine antigens were characterized with multiple different methods. Quality control analyses ensured the lack residual DNA and endotoxins.

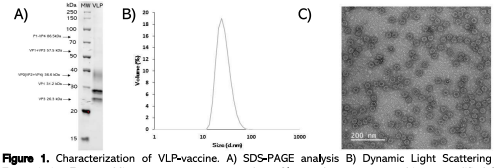
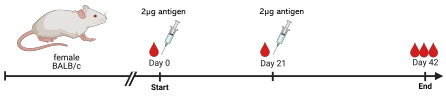


Figure 1. Characterization of VLP-vaccine. A) SDS-PAGE analysis B) Dynamic Light Scattering and C) Transmission Electron Microscopy analyses ensured >95% pure, homogenous and intact modified CVB-VLP particles with an average size of 30 nm.

STEP 2. IMMUNIZATION STRATEGY



We have optimized the immunization dose and frequency in previous pilot studies and here we wanted to evaluate the effect of commercial Adjuvant System 04 (AS04) and immunization route. AS04 consists of Aluminium Hydroxide (potent humoral adjuvant) and Monophosphoryl Lipid A (MPLA, potent cellular adjuvant). Subcutaneous immunization is routinely performed in preclinical evaluation of a vaccine candidate, but most vaccines in clinical usage are administered intramuscularly.

STEP 3A. HUMORAL IMMUNITY

Termination serum was collected from all individual mice and antigen-specific IgG and IgG subtype antibodies were determined with ELISA.

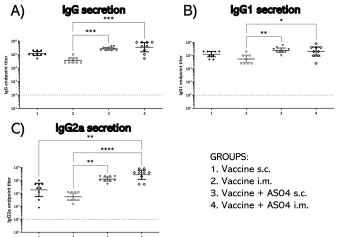
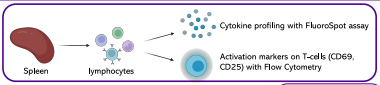


Figure 2. Vaccine antigen –specific A) IgG, B) IgG1 and C) IgG2a levels determined with end-point dilution from each individual mice. The positivity cut off (horizontal line) was measured with mice vaccinated with PBS+ AS04, counted as AVG OD+3*STD.

STEP 3B. CELLULAR IMMUNITY



Isolated splenocytes were used in two separate assays to determine the cellular immune responses.. We used FluoroSpot assay to determine cytokines secreted by the splenocytes after an active CVB1 stimulation. In addition, we used 12-dye FACS panel to profile the activation markers on T-cells stimulated by the vaccine antigen.

GROUPS:
1. PBS + AS04 i.m
2. Vaccine s.c.
3. Vaccine i.m.
4. Vaccine + AS04 s.c.
5. Vaccine + AS04 i.m.

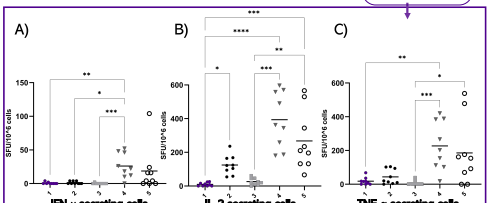


Figure 3. Spot- Forming units per million splenocytes determined with FluoroSpot analysis. A) IFN-γ, B) IL-2 and C) TNF-α secreting cells after stimulation of infective CVB1.

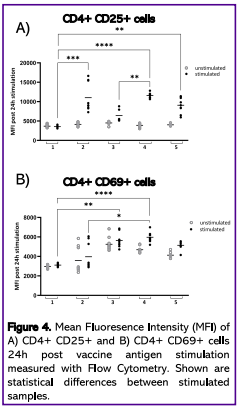


Figure 4. Mean Fluoresence Intensity (MFI) of A) CD4+ CD25+ and B) CD4+ CD69+ cells 24h post vaccine antigen stimulation measured with Flow Cytometry. Shown are statistical differences between stimulated samples.

CONCLUSIONS

Production of modified CVB-VLPs in insect cells was done successfully with abundant yields. The purified VLPs were extensively characterized, revealing homogenous and intact particle formation of the vaccine antigens. Adding a commercial adjuvant AS04 significantly increased the levels of antigen-specific IgG levels in the sera of vaccinated mice. Moreover, T-cell specific cytokine production of virus-stimulated splenocytes was elevated in the adjuvanted groups. Flow Cytometry data on CD4+ T-cell activation (as expression of CD25 and CD69 receptors) showed activation in all vaccinated groups but confirmed the effect of the adjuvant enhancement. Interestingly, the immunization route did not seem to have a statistically significant effect in any of the methods used to evaluate the immune response towards the modified CVB-VLP.

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