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Research Article

Lipid A mimetic BECC438 potentiates durable and balanced antibody responses in an ovalbumin murine model of vaccination

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Abstract

The need for effective infectious disease vaccines has become an inescapable topic in recent years. Continued development of next-generation vaccines that provide robust protective immunity is imperative. Such vaccines will likely include adjuvants that avoid excessive adverse reactions while allowing for dose and antigen sparing. Bacterially derived TLR4 agonist, BECC438, has recently emerged as a lead adjuvant candidate across several experimental models of infectious disease, including *Yersinia pestis* (plague), human papillomavirus, influenza A (flu), SARS-CoV-2 (COVID-19), and *Shigella* spp (gastrointestinal infection). To confirm that BECC438 is a high-quality immunoadjuvant, even without antigen from an infectious pathogen, studies presented here use the model antigen ovalbumin in a murine prime-boost vaccine model. Durable and more balanced production of antibody isotypes IgG1 and IgG2 is observed when the bacterial enzyme combinatorial chemistry adjuvant is used, as compared with the classic adjuvants aluminum salts (Alhydrogel) and synthetic monophosphorylated lipid A–PHAD (phosphorylated hexaacyl disaccharide). Antibody responses are maintained for at least 18 wk postvaccination. Observed immune metrics maintained similar trends across males, females, and genetic backgrounds, including C57BL/6, BALB/c, and CD-1 (outbred) mice, with males overall showing a lower production of IgG2c. In vitro analysis of C57BL/6 serum showed an increased half-life of ovalbumin-specific antibodies in BECC438 adjuvanted animals, indicative of a higher antigen binding affinity. These studies provide continued evidence to support the development of the BECC438 adjuvant in vaccines for human use.

Keywords: adjuvant, antibodies, lipopolysaccharide, Toll-like receptor 4, vaccination

Introduction

Adjuvants are a critical component of modern-day subunit vaccines, these immune-stimulating agents have the opportunity to tailor the adaptive immune response that is elicited by a vaccine.[1–3] Lipid A is a canonical ligand for the innate immune receptor Toll-like receptor 4 (TLR4), which, when activated, initiates the production of cytokines that can help shape subsequent adaptive immune responses.[4–6] Bacterial enzyme combinatorial chemistry (BECC) has allowed for the production of a library of bacterial lipid A with a diverse array of structures.[7] From these studies, BECC438 has emerged as a lead adjuvant candidate. Structurally, BECC438 is a bisphosphorylated hexa-acylated lipid A with primary C14 acyl chains attached at the 2, 3, 2′, and 3′ positions with a secondary C16 acyl group attached through an O-linkage at the 2 and 3′ positions.[8] When isolated from a biologic source, the lipid A structure has core and O-antigen subunits attached. These molecules are named lipopolysaccharide if repeating O-antigen subunits are included and lipooligosaccharide (LOS) if only the core oligosaccharides are attached via the 6′-hydroxyl of the lipid A diglucosamine sugar.[9] Chemical treatment using mild acid and washing can remove core and O-antigen sugars by hydrolyzing the bond attaching core oligosaccharide to lipid A.[10] Synthetic chemically synthesized lipid A does not have core and O-antigen attached. The BECC438 lipid A structure has been isolated and structurally characterized from both biologic and synthetic sources,[11] studies presented here are completed with the biologic form of LOS or lipid A.

BECC438 has been shown to provide superior protection in murine vaccination models for *Yersinia pestis*,[12] human papillomavirus,[8] influenza A,[13,14] SARS-CoV-2,[15,16] and *Shigella* spp.[17] The ability to drive cross protective responses against different strains of influenza in both adult and elderly aged mice highlights the broad applicability of BECC438. Using bacterial antigens from *Shigella* spp and *Y. pestis*, BECC438 has been found to induce cytokine profiles that are consistent with protective immune responses. Specifically, relatively high levels of interferon-γ and interleukin-17 induced by BECC438 were measured in

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challenged mice that had improved outcomes.[17,18] The route of administration must also be considered when working to define the efficacy and immune stimulatory profile of vaccines. Recently, the biologic form of BECC438 was found to preferentially induce T helper 1 (Th1) skewed immune responses when included in an intranasally delivered *Bordetella pertussis* vaccine as compared with intramuscular delivery.[19] This intranasal vaccine also provided superior protection from lung infection following respiratory challenge with *B. pertussis* and provides further evidence of the broad reaching usefulness of the BECC438 adjuvant against human pathogens. Chemical formulation strategies must also be considered when characterizing best ways to deliver vaccines, initial formulation studies for BECC438 show that oil-in-water emulsion strategies lead to highest efficacy.[17,20]

The repertoire of antigens for which vaccines are being developed continues to rapidly expand, now including not only infectious disease targets,[21,22] but also cancer associated[23–25] and autoimmune antigens.[26,27] As new antigens are identified for inclusion in vaccination strategies, the pathway to antigen presentation and cytokine response that is initiated must be considered.[28] Immune responses elicited by protein antigens are highly variable and depend largely on the cytokine milieu surrounding the antigen as it is presented to responding naïve B lymphocytes. If Th1-associated cytokines are present, B cells maturing to recognize the antigen are likely to isotype switch to produce IgG2. If cytokines produced by Th2 cells are present, the B cells will likely isotype switch to produce IgG1.[29] These immunoglobulin subtypes activate different downstream immune responses once they are bound to the antigen they are specific for. IgG2 is most effective at initiating immune responses geared toward neutralizing intracellular pathogens, and IgG1 is most effective against extracellular and parasitic pathogens.[29,30] Before isotype switching, competition among B cell clones in germinal centers drives production of higher affinity antibodies which bind to antigen with greater strength.[31] Adjuvants can work to provide more isotypic consistency of antibody immune responses among heterogeneous human populations and variable concurrent environmental stimuli.

Ovalbumin (Ova) is a model antigen that has been used to study specific immune responses involving antigen presentation, it is also an antigen that is known to be able to initiate allergic immune responses driven by IgE/IgG1 antibodies after sensitization.[32–34] Ova is frequently used as a model antigen in vaccine studies, often to characterize new delivery and formulation methods.[35,36] In the studies presented here, the model antigen ovalbumin is used to further define the quality of immune responses elicited by BECC438. Previous studies investigate the efficacy of BECC438 to protect from pathogens using infectious challenge and have a relatively short timeframe to endpoint of experiment. These studies seek to use the well characterized Ova antigen to ensure durability and quality of immune responses elicited when combined with the novel immunoadjuvant BECC438. Responses and observations made and attributed to BECC438 using the Ova protein were confirmed using the *Y. pestis*–derived bacterial antigen rF1V.[37,38] Taken together, immune responses measured using Ova and rF1V antigens will provide experimental evidence to further characterize BECC438-elicited antibodies without an infectious challenge endpoint.

Immune response variability between individuals is also affected by differences in biologic sex and genetic backgrounds. Experimental murine models often use genetically inbred mouse strains to lessen the effect of genetic variability in results. Two of the most commonly used genetic backgrounds are C57BL/6 mice, which are commonly known to be capable of producing Th1 immune responses, and BALB/c mice, which are known to preferentially produce Th2 immune responses.[39] The ability of BECC438 to induce immune responses in these backgrounds is investigated, and well as the CD-1 mouse background which is outbred and may be more indicative of the range of responses that are seen in human populations.[40] In the studies presented here, a murine model of prime-boost vaccination is used to determine the duration of antigen-specific antibodies, the antibody isotype ratios that are induced, the ability of the lipid A fraction to induce adjuvant properties, and the relative affinity of antigen-specific antibodies elicited. Overall, we conclude that, when compared with currently used adjuvants, BECC438 is capable of producing antibody responses that are similar or improved in isotypic balance, durability, and antigen binding affinity.

Materials and methods

BECC438 isolation and purification

BECC438 was isolated from a *Y. pestis* strain that was previously described in Gregg et al.[12] Growth conditions, LOS extraction, and mild acid hydrolysis were performed as previously described.[7,41] Purified LOS and lipid A were stored as lyophilized powder in a −20 °C freezer until reconstituted for use in immunizations.

Mouse immunization and blood collection

All mouse studies have been approved by the University of Maryland Institutional Animal Care and Use Committee Protocol #0918003. Six- to 8-wk-old mice were purchased from the Jackson Laboratory (C57BL/6) or Charles River (BALB/c and CD-1). Mice were injected intramuscularly in the caudal thigh on a prime (day 0) and boost (day 14) schedule with 50 µL containing described combinations of 50 µg of an adjuvant, Alum (Alhydrogel; InvivoGen; Cat# vac-alu), synthetic monophosphoryl lipid A (MPLA)–PHAD (phosphorylated hexaacyl disaccharide) (Avanti; Cat# 699800), or BECC438[12]; and an antigen, 10 µg of Endofit-Ovalbumin (InvivoGen; Cat# vac-pova) or 1 µg rF1-V fusion protein (*Y. pestis* F1-V Fusion Protein, Monomer-Enriched Antigen, Recombinant from *Escherichia coli*, NR-2561; BEI Resources). Upon resuspension, BECC adjuvants and PHAD were placed in a water bath sonicator for 15 min to promote micelle formation. Before injection adjuvant and antigen combinations were resuspended in sterile 1× phosphate-buffered saline (PBS) at described concentrations, they were mixed by vortex, and allowed to adsorb for 2 h at room temperature before injection. Blood was collected from the lateral saphenous vein on 2-wk intervals, before injection on days 0 and 14, and serum was isolated using Microvette Z-gel serum tubes (Sarstedt; Cat# 20.1344). Serum was placed in a sealed sterile 96-well plate and frozen at −20°C until use in enzyme-linked immunosorbent assay (ELISA) experiments.

Enzyme-linked immunosorbent assay

Indirect ELISAs were used to compare relative quantities of Ova-specific or rF1-V-specific antibody isotypes. Each well of a flat-bottom 96-well Nunc-Immuno Maxisorp plate

(Millipore Sigma) was coated with 10 to 100 ng of Endofit-Ovalbumin or rF1-V resuspended in 50 µL of sodium bicarbonate buffer, sealed, and incubated overnight at 4 °C. Wells were washed 3× with wash buffer (1× PBS + 0.05% Tween 20), then 50 µL of 1% bovine serum albumin in 1× PBS (bovine serum albumin Fraction V) was added to each well and the plate was sealed and incubated at 37 °C for 1 h. Murine serum was diluted in 1× PBS + 0.1% bovine serum albumin using a 5-point dilution curve. Blocking solution was removed from each well of the plate, 50 µL of the serum dilutions were added to the plate in duplicate, and the plate was then sealed and incubated at 37 °C for 2 h. Wells were washed 3× with wash buffer, 50 µL of a 1:4,000 secondary antibody solution in 1× PBS was added to each well, and the plate was sealed and incubated at 37 °C for 2 h. Secondary antibodies specific for murine antibody isotypes were used as follows: IgG1 (Southern Biotech; Cat# OB107005), IgG2c (Southern Biotech Cat# OB107705), and IgG2a (Southern Biotech; Cat# OB108005). The plate was washed 3× with wash buffer, developed with OptEIA TMB Substrate Reagent Set (BD Biosciences; Cat# 555214) following manufacturer instructions. The optical density at 450 nm was read for each well followed by data analysis completed in GraphPad Prism (version 8.4.3; GraphPad Software) using a 4-parameter logistic line of best fit.

Biacore

Off-rates and kinetic parameters of protein-protein interactions were measured by surface plasmon resonance immunogenicity analysis using a Biacore T100 biosensor (GE Healthcare). A total of 3,000 response units of Endofit-Ovalbumin were immobilized on flow cell 2 of a CM5 sensor chip; flow cell 1 was blanked and used as reference. 1:50 dilutions of serum (3 µL in 150 µL HBS-EP) were run on overflow cells and regenerated using 50 mM HCl. Chip integrity was routinely confirmed using a control sample for response unit generation. Sensorgrams were double-referenced against the control flow cell and buffer injections. Data were determined through immunogenicity binding models (50 response unit boundary) using Biacore T200 Evaluation Software. Total response units, percent fractions of fast/slow antibodies, and half-lives [$T_{1/2}$ (s) Slow, where ln2/k] were determined.

Results

BECC adjuvant initiates durable and robust antibody responses

It has already been established that BECC adjuvants are able to enhance immunity when included in intramuscular vaccination strategies. In the studies presented here, the model antigen, Ova, was mixed with and allowed to adsorbed to novel adjuvant BECC438, Alhydrogel, or PHAD. These inoculations were then administered intramuscularly in the caudal thigh to mice on a 2-wk prime-boost interval. To determine if antigen-specific antibody levels are maintained over time, serum was collected from the lateral saphenous vein for up to 18 wk postvaccination and antibody levels were then measured using an ELISA. In C57BL/6 mice, it is observed that Ova-specific antibody levels are maintained for the length of experiment with a slight decrease 12 wk following the first vaccination and 10 wk postboost (Fig. 1). IgG1 levels were the most robust, whereas IgG2c levels remained relatively low. For intracellular viral and bacterial antigens, having a more balanced ratio of IgG1 to IgG2 could contribute to greater protection, as compared with the characteristic high-level IgG1 response elicited by aluminum salts adjuvant. Mice vaccinated with BECC438-adjuvanted Ova show the lowest levels of serum IgG1 and BECC438, along with PHAD, elicits among the highest early levels of IgG2c with Alum inducing similar levels by the 8-wk time point.

Differences in antibody production between sexes have been found across a wide range of vaccination strategies.[42,43] To further define immune responses driven by BECC438, both male and female mice were inoculated with adjuvanted vaccines. Using Ova model antigen, no significant difference in antibody production was observed between males and females; however, there was a trend that males had attenuated IgG2 production as compared with females (Fig. 2). Although IgG2 antibody titer levels are lower in males, this disparity is smaller using inoculums adjuvanted with BECC438. To be able to make direct comparisons among groups, only female mice were used in the subsequent experiments.

Beyond differences in immune responses between sexes, the human population is genetically diverse, and it is important to be able to induce robust immunity in a wide range of genetic backgrounds. To begin to address this necessity, inbred C57BL/6, inbred BALB/c, and outbred CD-1 mice were vaccinated with Ova as described previously. As expected, BALB/c mice predominantly produced IgG1 regardless of which adjuvant was used, while C57BL/6 mice were more capable of producing IgG2 when combined with an adjuvant (Fig. 3). Intriguingly, the BECC438 adjuvant was able to initiate a more balanced production of IgG1/IgG2 in the genetically diverse outbred CD-1 mice as compared with Alum and PHAD. This is a notable finding given that the Ova protein is known to preferentially initiate allergic IgE/IgG1 antibody responses, and this predisposition was skewed toward production of IgG2 when BECC438 was included as an adjuvant.

To confirm that lipid A of the biologically derived BECC LOS is the portion of the molecule that is imparting adjuvant activity, mild acid hydrolysis was performed to isolate the lipid A from the O-antigen portion of the LOS molecule. Full-length LOS or the lipid A fraction were then adsorbed to antigen and injected intramuscularly in C57BL/6 female mice. Both the lipid A and LOS forms of BECC438 elicit similar IgG1 and IgG2c responses to the Ova (Fig. 4). Immune responses elicited by the *Y. pestis* rF1V antigen have also been well characterized while being developed for use in vaccines and shows capability to induce rapid protective immune responses.[44–46] To further support observations about the effectiveness of BECC438 at boosting immune responses elicited to the Ova antigen, similar experiments comparing the ability of BECC438 LOS and lipid A to stimulate antibody responses to the well characterized *Y. pestis* rF1V antigen were also completed. LOS and lipid A adjuvants were found to promote similar production of rF1V-specific antibodies (Fig. 4). Overall, the lipid A form of the BECC438 adjuvant induces a slightly lower average antibody production than the LOS form. However, this average value is reliably boosted by the second vaccine dose, and an antibody level increase is observed in the week 4 serum samples. Both Ova-specific and rF1V-specific levels of IgG1 and IgG2c were boosted following the second vaccine dose adjuvanted with

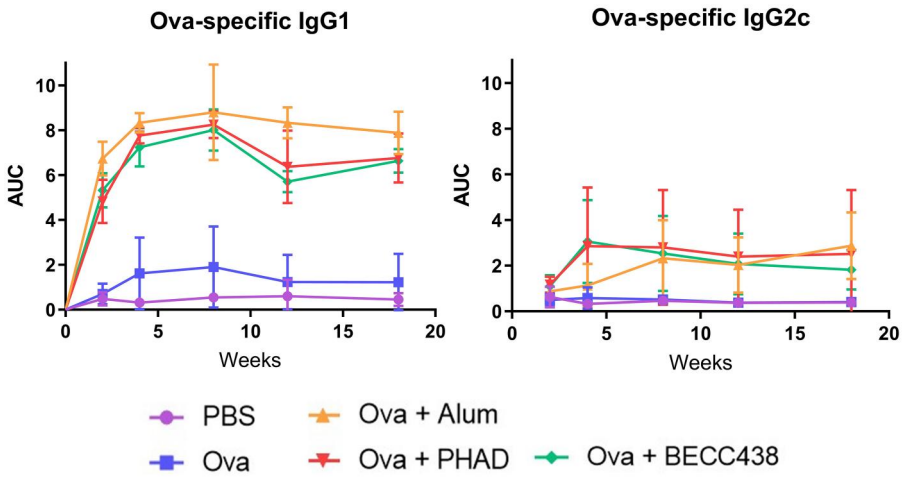

Figure 1. Durability of antibody immune responses initiated by vaccines adjuvanted with BECC438. C57BL6 male (n = 4) and female (n = 3) were inoculated intramuscularly on day 0 and day 14 with PBS (vehicle control), Ova alone, Ova+Alhydrogel, Ova+PHAD, or Ova+BECC438. Serum was collected on weeks 2, 4, 8, 12, and 18. Ova-specific antibody quantities were measured by indirect ELISA using a 5-point dilution curve. The area under the curve (AUC) for each sample is calculated in GraphPad Prism v7 based on a 4-parameter exponential line of best fit. The mean ± SD of combined results from males and females (n = 7 total for each group) is shown on graphs.

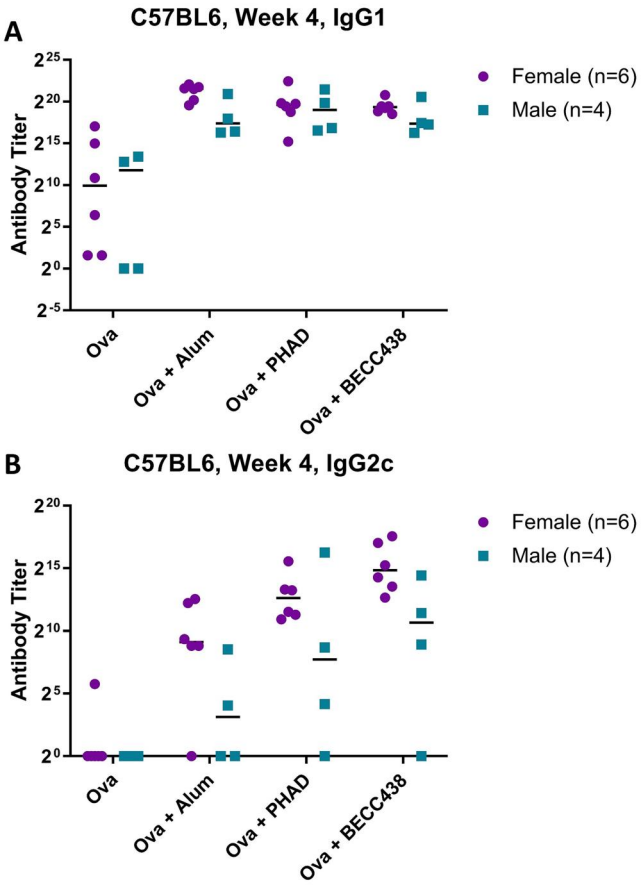

Figure 2. Comparison of IgG1 and IgG2c production between male and female mice. C57BL6 male (n = 4) and female (n = 6) mice were inoculated intramuscularly on day 0 and day 14 as previously described. Serum titers of Ova-specific IgG1 and IgG2c were measured using an indirect ELISA method. Data from the week 4 serum samples are pictured, and the individual biologic replicate and mean values of each experimental group are shown. Antibody titer is defined as the serum dilution at which the a 4-parameter exponential line of best fit equals 2× the background level for the plate on which the sample was measured. Titer determination and graphs were made using GraphPad Prism v7.

either BECC438 LOS or BECC438 lipid A indicating that the minimal lipid A portion alone is imparting adjuvant activity.

Acute toxicity is not observed in New Zealand white rabbits

To enable BECC438 to be eligible for use in human clinical trials, potential to cause acute toxicity must be tested. New Zealand white rabbits are the most commonly used experimental model to measure metrics of acute toxicity of therapeutic agents. Male and female rabbits were administered the vehicle control article or 50 or 100 µg/dose BECC438 via intramuscular injection once on day 1 of the dosing phase (Table 1). A BECC438-related significant increase in mean body temperature was seen in both sexes administered ≥50 µg/dose at 2 h postdose, 39.8 °C in males and 40.0 °C in females. A significant increase in body temperature was observed at 4 h postdose in males (40.4 °C) and females (39.7 °C) administered 100 µg/dose. An increase was also observed on day 3 of the dosing phase in females administered 50 µg/dose. Due to the transient increase in body temperature and absence of reactogenicity, 100 µg/dose was considered well tolerated. All animals survived to their scheduled sacrifice. No remarkable clinical observations were noted; no alterations in body weight, body weight changes, food consumption, dermal observations, or macroscopic or microscopic observations were noted at necropsy. Experiment conducted and samples analyzed by Covance (Covance/Labcorp Study 8422765).

BECC438 drives increased antigen specific antibody affinity

Although having a greater amount of antigen-specific antibodies correlates with providing more robust immunity against the target antigen, it is also critical to see how strongly these antibodies bind to the target antigen. Having a higher antibody binding affinity also correlates positively with the level of protection that is elicited.[47] ELISA assays accurately determine the amount of antigen-specific antibodies, but they are based on saturated endpoint measures, so the binding strength of the antibodies is not readily determined.

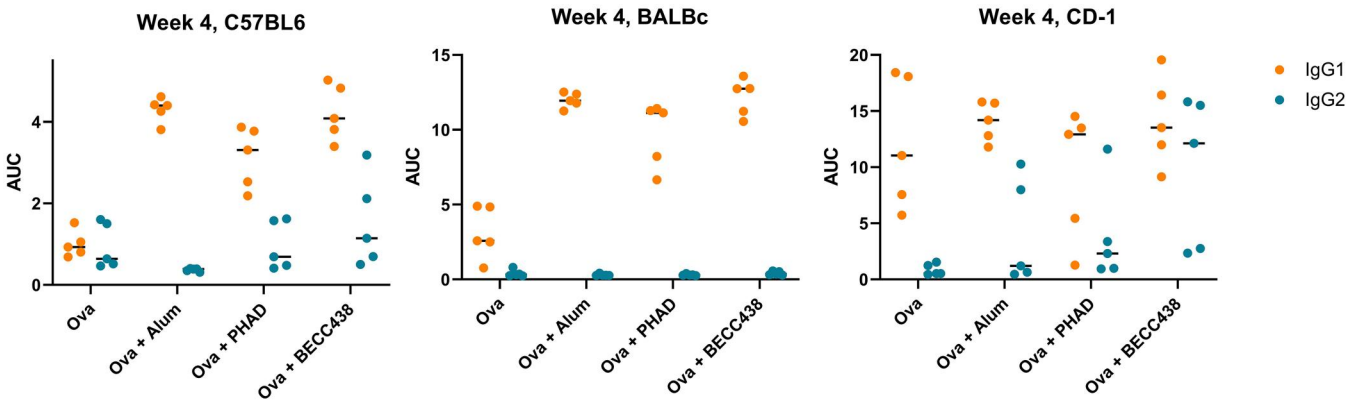

Figure 3. Consistent responses are elicited across genetic backgrounds. Female (n = 5) mice of C57BL6, BALB/c, or CD-1 background were inoculated as described in the methods on day 0 and day 14. The area under the curve (AUC) was calculated in GraphPad Prism v7 based on a 4-parameter exponential line of best fit is shown on the graphs. Data from the week 4 serum samples are pictured, and the individual biologic replicate and mean values of each experimental group is shown.

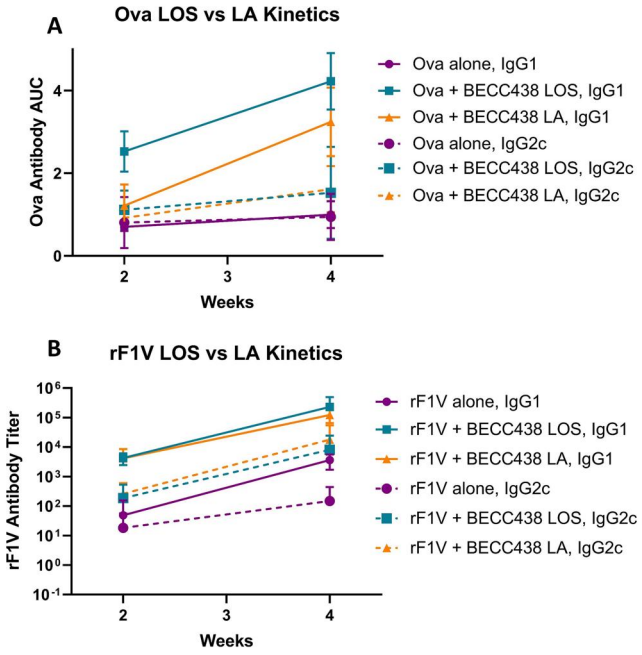

Figure 4. LOS versus lipid A induced similar magnitude antibody responses. C57BL6 female mice (n = 5) were vaccinated using 50 μg of adjuvant combined with (A) Ova or (B) rF1V protein and serum antibody amounts were measured using an indirect ELISA method. For the Ova protein, the area under the curve (AUC) was calculated based on a 4-parameter exponential line of best fit is shown on the graphs. For the rF1V protein, the antibody titer was defined as the serum dilution at which the a 4-parameter exponential line of best fit equals 2× the background level for the plate on which the sample was measured. Titer determination, AUC, and associated graphs were completed using GraphPad Prism v7.

The polyclonal and multi-isotypic nature of in vivo immune responses make it technically difficult to directly measure antibody affinity from serum. Here we use a Biacore immunogenicity assay to measure antigen-specific relative antibody affinity from postvaccine mouse serum. In the first measure tested, response units of antibodies bound to Ova protein, BECC438, Alhydrogel, and PHAD are found to elicit a similar amount/concentration of Ova-specific antibodies (Fig. 5A). At week 3, BECC438 is found to elicit a higher amount of response units as compared with Alhydrogel and PHAD, and this equates to higher relative amounts of antigen-specific antibodies at the earlier time point. In addition, the average binding half-life of these interactions show that antibodies elicited by BECC438-adjuvanted inoculations also have a similar but slightly higher relative affinity for the target antigen (Fig. 5B). Overall results provide evidence that BECC438 is able to drive the production of a high concentration of high-affinity antibodies and remains to be a top-tier candidate for inclusion in component vaccine formulations.

Discussion

Developing an effective vaccine begins with identifying an antigenic protein that contains epitopes for which generation of antibodies will provide protection from infection. Once this is accomplished, route of administration, formulation, and use of an immunoadjuvant must be considered. Previous studies have determined the effectiveness of experimental adjuvant BECC438 to initiate protective immune responses from infectious challenges. The success of these studies has allowed for these immunoadjuvants to be included in pre–investigational new drug studies and has created the need determine if BECC438 induces antibody responses that are durable beyond the infectious challenge endpoints.

To be confident in investing the resources necessary to continue developing the adjuvant structure BECC438 for use in human vaccine trials, immune response durability, quality, broad applicability, and potential reactogenicity must be determined. As a part of the AS04 vaccine adjuvant formulation, the lipid A adjuvant already approved for clinical use, MPLA, has been credited with contributing to the high efficacy of the human papillomavirus vaccine, Cervarix, and the hepatitis B vaccine, Fendrix. BECC438 has a similar hexaacylated structure as MPLA; however, BECC438 is bisphosphorylated instead of monophosphorylated. PHAD is a synthetic structural analog of MPLA that has also been shown to be an efficacious vaccine adjuvant. Approval of MPLA for use in vaccine formulation paves the way for the use of other lipid A mimetics, such as BECC438, for in vivo use. The results presented in this study identify that the magnitude of antibody response is similar to the well-characterized adjuvants Alum and PHAD, but the antibody response from BECC438 adjuvanted vaccines is overall more isotypically balanced and has a higher antigen-specific relative binding affinity. As shown in Fig. 2, BECC438 had the most balanced

Table 1. Toxicity study in New Zealand White rabbits.

Group[a]	Animals		Dose Level (μg/dose)	Dose Concentration (μg/mL)[b]	Dose Volume (mL/animal)
	Male	Female			
1. Control	3	3	0	0	0.20
2. Low	3	3	50	500	0.10
3. High	3	3	100	500	0.20

A toxicity study using New Zealand white rabbits was conducted by Covance in the Covance/Labcorp Study 8422765 with parameters as described in this table.
[a] Group 1 was administered vehicle control article only.
[b] Dose concentrations were based on test article supplied. No correction factor was used.

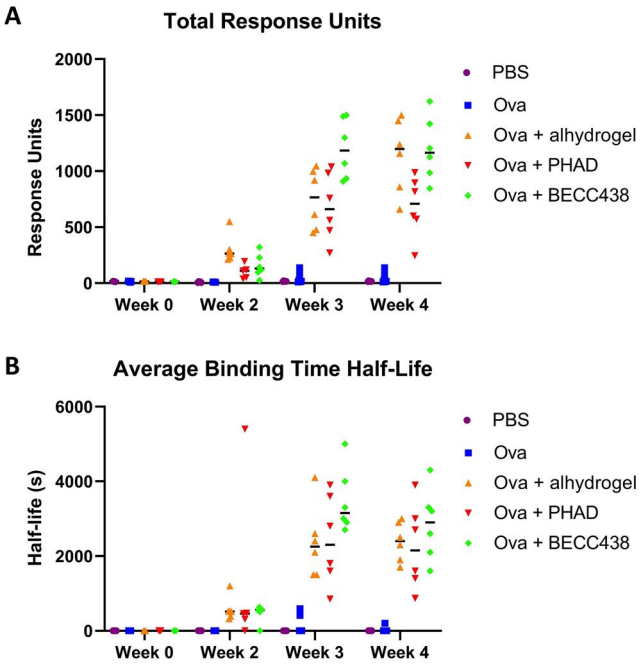

Figure 5. Serum antibodies from BECC438 adjuvanted mice elicit high relative antigen binding affinity. Mouse serum (n = 6 female C57BL6) from the experiment described in Fig. 3 was utilized in a Biacore immunogenicity assay. For this Ova is amine coupled to a CM5 chip and serum ran over at 1/50 dilutions. The off-rate ($T_{1/2}$) was not concentration dependent and was used as a comparator metric to determine antibody affinity. Depicted are (A) the total response units (mean marked) and (B) the half-life of the response unit antibodies for each experimental group (mean marked).

isotype production across sexes. This relatively more balanced profile of antibody isotypes is a positive indicator of success because one of the main goals in developing BECC adjuvants is to initiate more balanced Th1 and Th2 immune responses. Serum samples from this study were also tested for the levels of several different Ova-specific antibody isotypes, including IgG2b and IgG3; however, the levels of these isotypes were below the limit of detection of the assays conducted. Recent characterization of immune responses initiated in a DTaP vaccine model adjuvanted with BECC438 highlight unique immune responses elicited with an intranasal route of administration versus an intramuscular injection.[19] Additional experiments with a larger repertoire of antigens, mouse strains, administration routes, and detection methods will be necessary to define the full antibody isotype profile that is elicited by the BECC438 adjuvant.

Because the lipid A portion and lipopolysaccharide stimulate similar responses (Fig. 3), chemically synthesized lipid A with the same structures will likely be capable of initiating similar immune responses. This chemical synthesis allows for easier production of GLP adjuvants without concern of possible bacterial contaminant residue from the lipid A extraction process. These vaccine inoculations were prepared using simple adsorption and are from a biologic source, and formulation and synthetic manufacturing will help to optimize effectiveness of the adjuvant molecule BECC438.[48] Chemical characterizations of commonly used formulation strategies with both the biologic and synthetic versions of BECC438 have recently been reported[11] and provide proof of principle for the use of these synthetic analogs in future. The BECC438 adjuvant initiates production of antigen specific antibodies that have a relatively increased binding half-life (Fig. 4). These antibody binding affinities are emerging as an important indicator of efficacy,[49] and future studies will use more nuanced vaccine formulation to investigate the ability of BECC438 to promote even higher-quality antibodies. Although the immunogenicity of BECC438 has been characterized using ex vivo and in vitro human cells,[12] future studies should be conducted in other animal models, including nonhuman primates. These will serve to confirm that observations made in murine models will carry through when the adjuvant is used in vivo in human populations. It will also be important to continue with the determination of safety using additional toxicity models. The ability of BECC438 to potentiate quality durable immune responses in multiple genetic backgrounds and with a wide variety of antigens provides strong support for the use of these lipid A memetics in subunit vaccines that are currently under development for use in humans.

Author contributions

Conceptualization: E.M.H., R.K.E.; Formal analysis: E.M.H., J.F.K., D.J.V., G.A.S.; Investigation: E.M.H., J.F.K., J.C.H., D.M.O., D.J.V.; Supervision: E.M.H., E.J.S., G.A.S., R.K.E.; Methodology: J.F.K., G.A.S.; Writing – original draft: E.M.H., J.F.K.; Writing – review & editing: E.M.H., J.F.K., F.M.G.; Project administration: F.M.G.; Funding acquisition: R.K.E.

E.M.H. (Conceptualization [Lead], Formal analysis [Lead], Investigation [Lead], Supervision [Lead], Writing—original draft [Lead], Writing—review & editing [Lead]), J.K.F. (Formal analysis [Lead], Investigation [Lead], Methodology [Lead], Writing—original draft [Supporting], Writing—review & editing [Supporting]), J.C.S.H. (Investigation [Supporting]), D.M.O. (Investigation [Supporting]), F.M.G. (Project administration [Lead], Writing—review & editing [Supporting]), D.J.V. (Formal analysis [Lead], Investigation

[Lead]), E.J.S. (Supervision [Supporting]), G.A.S. (Formal analysis [Supporting], Methodology [Supporting], Supervision [Supporting]), and R.K.E. (Conceptualization [Lead], Funding acquisition [Lead], Supervision [Lead], Writing—review & editing [Lead])

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Conflicts of interest

R.K.E. has served on the board for and owns stocks in TollereBio Corporation; has patents 10358667 and 11124815 pending to TollerBio Corporation; and is the principal investigator of the National Institute of Allergy and Infectious Diseases–funded Broad Agency Agreement.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

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