Evaluation of the effect of a panel of adjuvants on immune response to norovirus virus-like particle vaccine in mice

Antonia Thomas, Lauren A. Ford-Siltz, Kentaro Tohma, Gabriel I. Parra

Division of Viral Products, Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA), Silver Spring, USA

Abstract

Norovirus is the leading cause of gastroenteritis resulting in 200,000 annual deaths globally. The extreme diversity presented by noroviruses presents a challenge for vaccine development. The leading vaccine candidate is an adjuvanted virus-like particle (VLP) composed of two variants. By themselves, VLP subunit vaccines are poorly immunogenic and therefore adjuvants are included in the formulation to promote a more rapid immune response, reduce the number of doses, and induce cross-protection across multiple variants. In this study, we investigate the effect of adjuvants on antibody mediated immune response to norovirus VLPs.

Methods: Groups of BALB/c mice (n=5) were intramuscularly immunized with 3 doses at 4 weeks apart with a wild-type GII.4 VLPs alone or in the presence of monophosphoryl lipid A (MPLA), Poly IC, AddaVax, Alum alone, Alum with MPLA, or Complete Freud's Adjuvant (CFA) adjuvants. The immune response in immunized mice was characterized by measuring total norovirus-specific binding antibodies, the homotypic and heterotypic histo-blood group antigen (HBGA) blocking antibodies, and by mapping the antibody repertoire to the major antigenic sites.

Results: Compared to non-adjuvanted vaccine, CFA adjuvant induced the highest norovirus-specific binding titers followed by Alum with MPLA, AddaVax, Alum, and Poly I:C, while MPLA alone showed a contraction in both binding and HBGA-blocking titers. Animals immunized with CFA, AddaVax, Alum with MPLA, but not Alum alone had more than a 2-fold increase in blocking geometric mean titer and a significant increase in the breadth of HBGA-blocking against other GII.4 variants as compared to non-adjuvanted vaccine. Mapping the epitope-binding repertoire by using mutants to a conserved epitope showed that AddaVax, Alum with MPLA, and CFA adjuvants provided greater HBGA-blocking titers than the wild-type VLPs used for vaccination.

Conclusion: The adjuvant dependent increase in binding and HBGA-blocking titers observed in the AddaVax, Alum with MPLA, and CFA vaccines correlates well with depth and breadth of cross-reactivity. Abolish binding of specific antibodies to a conserved site resulting in enhance HBGA blocking suggests that antibodies to conserved epitopes could obstruct binding of protective antibodies. Rational-manipulation of antigens could enhance protection and move us towards a universal norovirus vaccine.

Introduction

With hundreds of thousands of deaths world-wide caused by norovirus infection and the periodic emergence of distinct norovirus variants driven by changes in the VP1 protein over the last 15 years (Tohma K. et. al. mBio 2019), the need to for a universal norovirus vaccine that is protective against the many viral variants could not be greater. Towards this goal, to improve the immunogenicity of GII.4 VLP vaccine we investigate whether adjuvants will increase the depth and breadth across other GII.4 variants.

Once in the body a pathogen or vaccine will display pathogen associated molecular pattern (PAMPS) that interact with pattern recognition receptors (PRR) on innate immune cells inducing an immune cascade of chemokines and cytokines that increase antigen presenting cells (APC) promoting antigen uptake. APC will present antigens to naïve T cells which will differentiate into a Th1 cell for a cellular response or a Th2 cell for a humoral response. Adjuvants are meant to enhance immunogenicity for antigens that induce limited immune pathways, like recombinant proteins or virus like particles (VLPs), by inducing a different or multiple immune pathways. Here we test adjuvants that represent the induction of different immune pathways to assess which is best at enhancing a homologous and heterologous immune response.

Methods: Immunization scheme

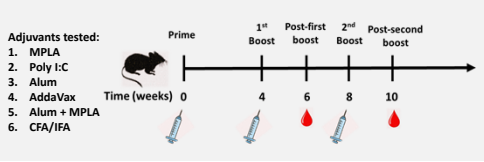


Figure 1. BALB/c mice (n=5) were intramuscularly primed with GII.4 Sydney 2012 (RockvilleD1 strain) VLP alone or in the presence of an adjuvant, then boosted twice four weeks apart. Serum was collected two weeks post first and second boost to assess the effect of adjuvants on antibody mediated immune response to norovirus VLPs.

Results and Discussion

A combination of first and second-generation adjuvants significantly enhance immunity to GII.4 VLP vaccine

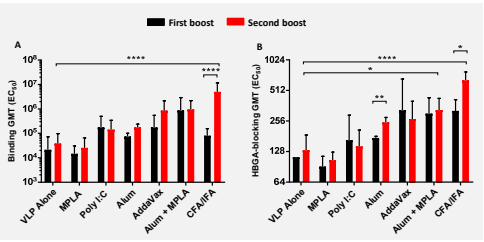
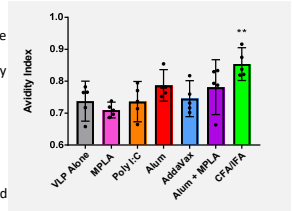


Figure 2. Serum collected two weeks post-first and -second boosts were used to assess B cell mediated responses. A) ELISA binding titer of RockvilleD1-specific antibodies. B) Histo-Blood Group Antigen (HBGA) carbohydrate blocking titer, a surrogate for neutralization, of RockvilleD1-specific antibodies. Geometric mean titers (GMT) and 95% CI (error bars) are represented. A t-test analysis was used for mean comparisons. *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$

CFA/IFA adjuvanted GII.4 VLP vaccine enhances IgG affinity maturation

Figure 3. Antibody affinity maturation was determined by measuring antibody binding in the absence and presence of chaotropic agent (urea). Antibody avidity was determined using the ratio of the area from the curves with and without urea treatment for each mouse sera. Mean avidity index and 95% confidence intervals (error bars) are represented. A t-test analysis used to compare means to VLP alone. **, $p < 0.01$



Regardless of the adjuvant, a GII.4 VLP vaccine is skewed towards a Th2 response

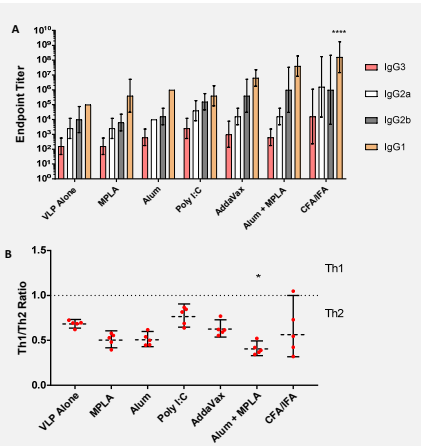


Figure 4. (A) IgG isotype titers were determined for terminal sera for each group analyzed. (B) Balance of the Th1/Th2 response was assessed by the ration of IgG2a/IgG1 isotypes. Shown are the geometric means and 95% confident intervals (error bars). A t-test analysis comparing means to VLP alone group. *, $p < 0.05$; ***, $p < 0.001$

Combinational adjuvants elicit significant depth and breadth in the cross-reactive response of GII.4 variants

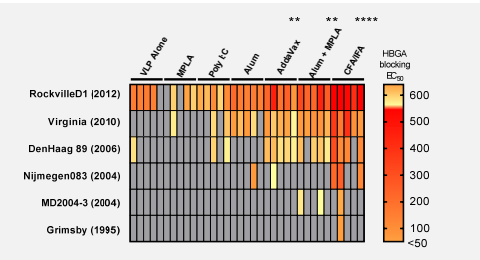


Figure 5. HBGA-blocking titer from mice sera post-second boost with or without adjuvant measured against the homologous RockvilleD1 vaccine stain and heterologous Virginia, DenHaag, Nijmegen, MD2004-3, and Grimsby strains (parenthesis represents the year of virus collection). Blocking titer for each mouse is colored as shown in the scale. Grey indicates a titer below the limit of detection (<50 EC_{50}). Mann-Whitney rank sum test analysis for treatment cross-reactivity across all virus variants as compared to the VLP alone group. **, $p < 0.01$; ****, $p < 0.0001$

Mutant VLPs to abolishing epitope binding antibodies

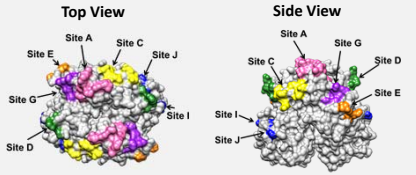
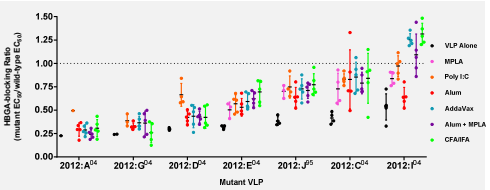


Figure 6. Antigenic sites of major capsid protein (VP1) from Rockville D1 vaccine strain from 2012 were swapped with corresponding antigenic site from MD2004-3 strain from 2004 (sites A, C, D, E, G and I) or Grimsby strain from 1995 (site J) previously confirmed monoclonal antibodies for each epitope.

Adjuvants enhance HBGA- blocking with loss of antibody binding to specific antigenic sites



Compared to VLP alone, which induces a more balanced immune response to all epitopes, the addition of adjuvants enhances HBGA-blocking with loss of anti- D, E, J, C and I antibodies suggests that adjuvants are not driving blocking with these epitopes and confirming an enhanced immunodominance for site A and G.

In the presence of combination adjuvants AddaVax, Alum with MPL, and CFA loss of the anti-I antibodies improves blocking beyond the wild type virus, suggesting that binding of anti-I antibodies is detrimental for immune protection against GII.4 noroviruses.

Figure 7. HBGA-blocking titer from mice sera post-second boost with or without adjuvant measured against mutant VLPs with antigenic sites swapped between RockvilleD1 strain and MD2004-3 or Grimsby strain. HBGA-blocking is represented as the ratio of HBGA blocking titer for mutant virus from the parental wild type virus. HBGA-blocking ratio of 1 is the level of blocking for wild-type RockvilleD1 strain.

Conclusion

- Adjuvants that induce multiple immune pathways to induce both a Th1 and Th2 response correlate well with depth and breadth of cross-reactivity.
- Combinational adjuvants confer enhanced immunity by targeting immunodominant sites while dampening non-dominant antigenic sites.
- Rational-manipulation of antigens could enhance protection and move us towards a universal norovirus vaccine