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Design and testing of mosaic enterovirus vaccine to prevent hand, foot and mouth disease

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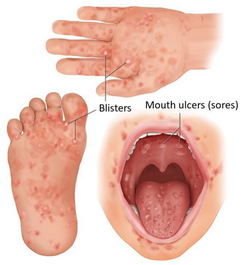
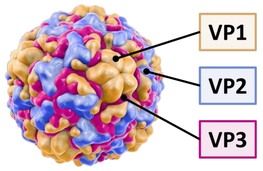
Hand Foot and Mouth Disease (HFMD) is caused by Enteroviruses

Hand, foot, and mouth disease (HFMD) is a common infection caused by members of the *Enterovirus* (EV) genus. HFMD predominantly affects children under 5 years old and presents with fever and rash. Although most cases resolved on their own, severe illness makes up 1.4% of cases and includes complications involving the central nervous system such as encephalitis and aseptic meningitis. Emergent EV strains could evolve to have enhanced infectivity and severe disease in future outbreaks.

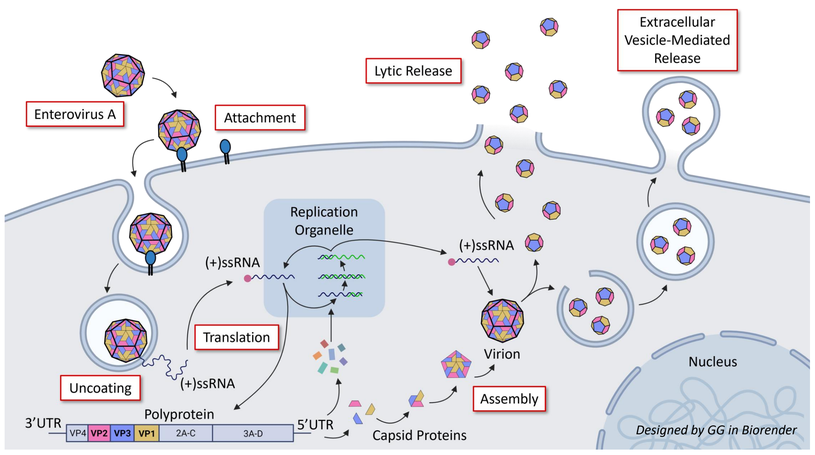


From infomedhealth.org



From observatorio.medicina.uc.cl

EVs are small, non-enveloped, viruses surrounded by a 30 nm diameter icosahedral capsid with a positive-sense single-stranded RNA genome. The genome encodes for 4 structural capsid proteins: VP1, VP2, VP3, and VP4. VP1 is the most external, surface-accessible capsid protein that serves as the enterovirus' viral attachment protein. Mutations in VP1 are responsible for changes in virus-receptor binding ability and virulence; thus, VP1 is a target for vaccine development.



Enteroviruses (EV) are a common cause of seasonal infection and can cause a wide spectrum of illness including HFMD. All EV serotypes known to infect humans are categorized into one of four species: EV A, B, C, or D. EV-A serotypes, of which ~20 infect humans, make up ~17% of all reported EV cases and are most associated with severe illness.

Currently, there are no vaccine or effective antiviral treatments against diseases caused by EV-A serotypes, primarily because of their diversity, limited cross-reactivity between epitopes, and rapid mutation rate.

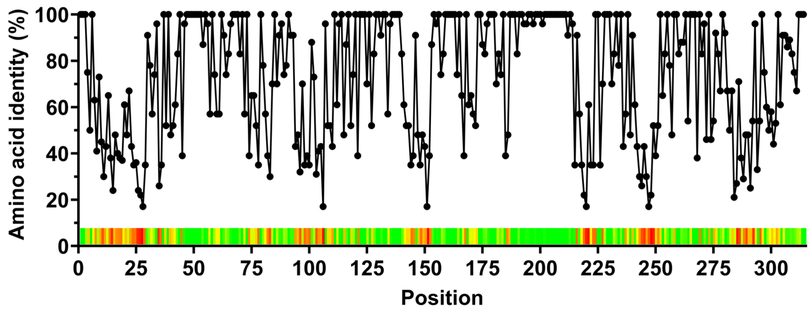
Purpose of Study

Design mosaic enterovirus VP1 immunogens to increase the breadth and depth of immune responses as compared to consensus or native enterovirus immunogens.

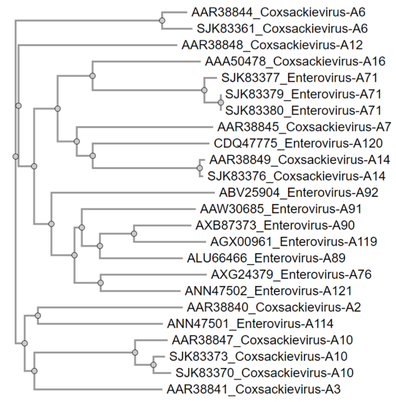
EV-A-Based Consensus & Mosaic Proteins

We used 24 EV-A VP1 sequences to build 5 mosaic VP1 proteins, as well as a consensus strain VP1. Across the alignment of the 24 natural sequences, 9-mer coverage increases with pentavalent and hexavalent configurations.

Sequence Conservation of 24 EV-A VP1 Proteins



24 Enterovirus-A VP1 Proteins



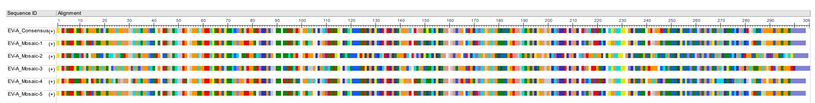
Consensus Protein

Recombinant protein with the most common amino acid at each position across an alignment of sequences

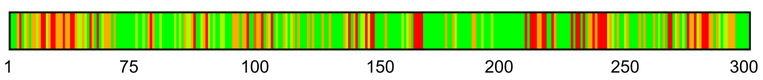
Mosaic Protein

Computationally-derived recombinant protein optimized for maximum inclusion of potential T-cell epitopes

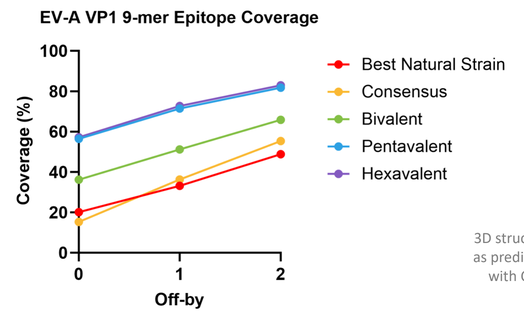
Amino Acid Sequence of EV-A VP1-based Consensus and Mosaic Proteins

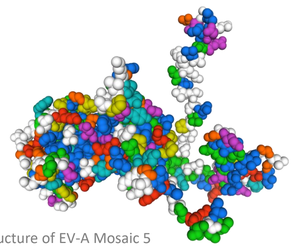


Sequence Conservation amoung Enterovirus A VP1 Mosaics



EV-A VP1 9-mer Epitope Coverage

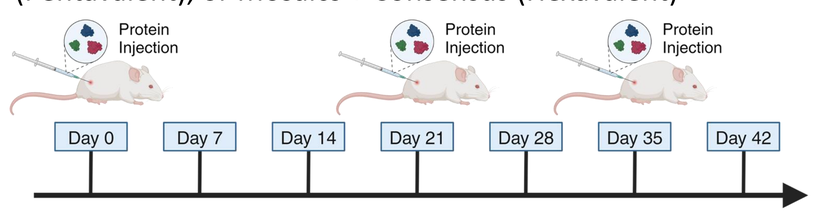




3D structure of EV-A Mosaic 5 as predicted by SWISS-MODEL with Clustal color scheme

In Vivo Injection of EV-A VP1-based Proteins

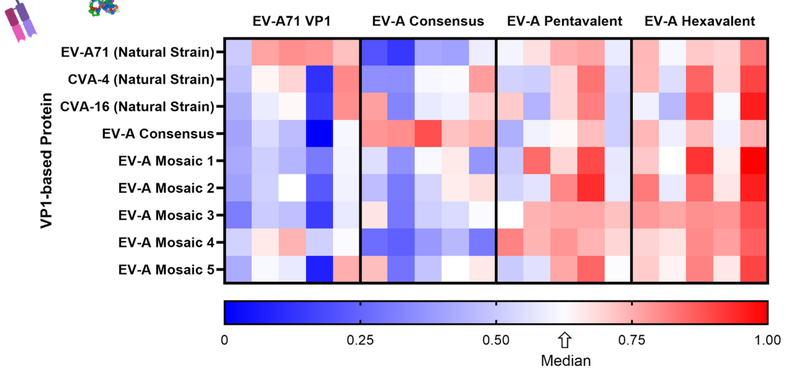
Female Balb/c mice (n=20) were given three I.M. injections of 17.5 µg of total protein in 50 µL 1X PBS and 50 µL Addavax over a 42-day period. Injections consisted of either EV-A71 VP1 (Natural Strain), EV-A VP1 Consensus, EV-A VP1 Mosaics (Pentavalent), or Mosaics + Consensus (Hexavalent)



Mosaic Vaccines Induce Antibody Response

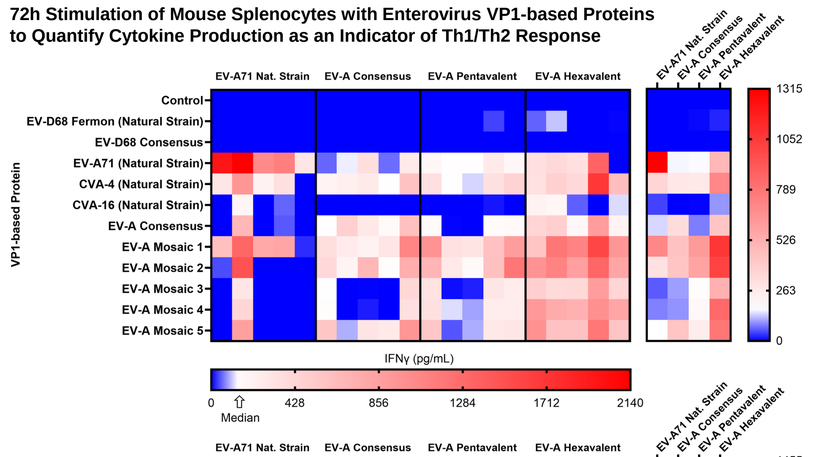
Serum from cardiac puncture blood was isolated for ELISAs. Indirect ELISAs were performed using EV-A VP1 or VP1-based antigens. Results indicated that mice injected with the hexavalent vaccine (5 mosaics + consensus) had an overall greater antibody response to the tested proteins.

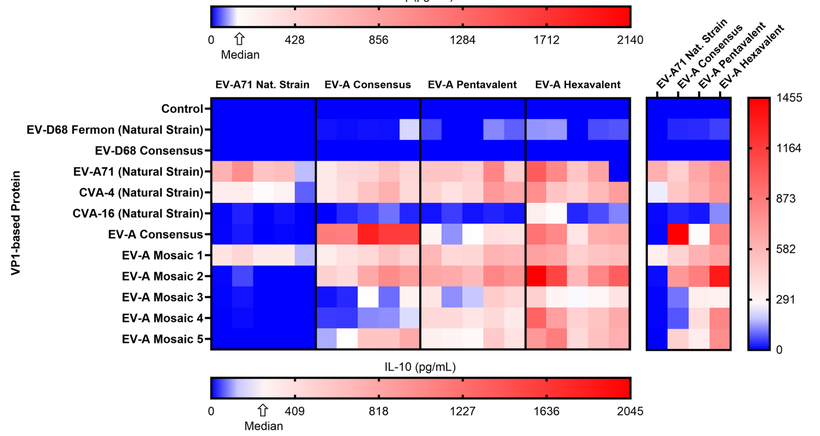
Normalized Individual Z-Scores of Antibody Binding
Antibodies from Cardiac Puncture Serum of Immunized Mice



Hexavalent Combo Induces T-Cell Response

72h Stimulation of Mouse Splenocytes with Enterovirus VP1-based Proteins to Quantify Cytokine Production as an Indicator of Th1/Th2 Response





Conclusions and Future Directions

Mosaic and hexavalent combinations induced antibody and T-cell response. Future studies will make use of enterovirus-based virus-like particles to induce neutralizing antibodies.

Acknowledgements

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