

ERVEBO Ebola vaccine will create a rice allergy epidemic, add to numerous autoimmune diseases, cancer and make Ebola disease even more severe. Design for safety and vaccine safety regulation remain abject failures. Incompetence or indifference?

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Abstract

The ERVEBO vaccine manufactured by Merck, contains residual rice proteins. Recombinant rice is used to manufacture human serum albumin present in the vaccine. Presence of residual rice proteins in the vaccine makes it immediately obvious that the vaccine maker and regulators are incompetent or indifferent about vaccine safety.

Injecting an alien protein induces IgE mediated sensitization directed against that protein. This vaccine will create an epidemic of IgE mediated rice allergy among recipients. A rice allergy epidemic in impoverished nations where rice is a staple food, will create a disaster that will dwarf any Ebola outbreak. Anaphylactic reactions to a common food like rice, lack of immediate access to medical facilities that can treat it and inability to afford personal emergency medications such as epinephrine injections, will make this a humongous disaster.

Rice proteins, other residual proteins (African green monkey proteins from VERO cell culture medium), vesicular stomatitis virus (VSV) proteins from the backbone virus and the envelope glycoprotein of the Zaire ebolavirus can all produce cross reacting immune responses against human self proteins. Bioinformatics analysis identifies numerous potential diseases due to such cross reactivity.

The same type of vaccine (live chimeric virus) was used for the dengue vaccine, Dengvaxia. So there is no reason to expect that the Dengvaxia disaster will not be repeated in this case.

This vaccine is only useful for limited administration in “ring vaccination” strategies to contain an Ebola outbreak. This vaccine is unacceptable and unsafe for prophylaxis in the general population using mass vaccination.

Introduction

ERVEBO is a chimeric virus based live vaccine. The vesicular stomatitis virus (VSV) envelope glycoprotein (GP) was replaced with the envelope glycoprotein from the Zaire ebolavirus. The final vaccine contains the live virus (and therefore all its proteins) as well as residual proteins from VERO (African green monkey derived) cell culture and rice proteins from the medium used to produce human serum albumin for the vaccine (1). These are the declared proteins and there are likely more undeclared proteins as well. So we have a product containing unknown quantities of tens of thousands of non-target proteins. An influenza vaccine was found to contain at least 293 chicken proteins, for example (2). Of course, this problem is not unique to the ERVEBO vaccine. In fact, the problem is not even

limited to vaccines but affects biologics such as monoclonal antibody treatments (3) and injected insulin (4). Yeast protein containing insulin injections predictably induce anti-saccharomyces cerevisiae antibodies (ASCA) (5). ASCA cause numerous autoimmune disorders (6).

Nobel laureate Dr. Richet warned us not to inject alien proteins into humans (7). Ignoring that and injecting tens of thousands of proteins into millions of humans, rises to a level of insanity that cannot be described in words.

This vaccine will create an epidemic of IgE mediated rice allergy among recipients (8–11). A rice allergy epidemic in impoverished nations where rice is a staple food, will create a disaster that will dwarf any Ebola outbreak. Anaphylactic reactions to a common food like rice, lack of immediate access to medical facilities that can treat it and inability to afford personal emergency medications such as epinephrine injections, will make this a humongous disaster.

VSV natural infection is rare in humans (12). So we don't know the potential for VSV infection induced autoimmune diseases. But infecting millions with VSV is now approved? VSV has only six proteins listed in UniProt (13). What happens when tens of thousands of residual proteins are injected along with the infectious agent? One can expect immune responses against ALL these residual proteins. So we need an autoimmunity analysis for tens of thousands of proteins.

Products must be designed for safety (14). Vaccines are developed using trial and error with no understanding of the immunological mechanisms involved (15,16). So they are unsafe by definition. With ERVEBO, Merck has continued that tradition of developing unsafe vaccines.

Wraith et al. (17) and Verdier (18) have suggested bioinformatics analysis during vaccine development to check for autoimmunity potential. They have also suggested autoimmune serology during clinical trials (19). Merck has done neither. CHOPPI (20) was developed to handle the equivalent problem with biologics.

We have known for at least twenty years that immunization with homologous xenogenic antigens results in the development of autoimmune diseases (21). Merck has ignored all of this.

We will therefore perform the analysis Merck has refused to do and demonstrate the danger the vaccine poses.

Methods

Basic local alignment search tool for proteins (BLASTP) (22), Universal Protein Resource (UniProt) (13) and the Immune Epitope Database (IEDB) (23) were used for bioinformatics analysis.

This limited analysis will predict a subset of diseases the vaccine will cause.

All proteins in the rice proteome were analyzed against the human proteome for sequence homology.
All proteins in the VSV proteome were analyzed against the human proteome for sequence homology.
Zaire ebolavirus GP was analyzed against the human proteome for sequence homology.
All human autoimmune disease related epitopes in IEDB (23000+ epitopes) were analyzed against African green monkey proteome for sequence homology.
All human autoimmune disease related epitopes in IEDB were analyzed against the rice proteome for sequence homology.

Human carbonic anhydrase II was analyzed against the rice proteome for sequence homology.

Proteomes and protein sequences were obtained from UniProt.

Results

Top human protein match results comparing with the African green monkey proteome

Immune responses against these antigens may result in autoimmune disorders that are yet to be discovered.

Accession number	BLASTP score	Antigen
NP_001677	869.304	ATP synthase subunit beta, mitochondrial precursor
AAH11384	705.157	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle
AEO89986	225.016	NADH dehydrogenase subunit 5 (mitochondrion)
BAH13528	196.174	unnamed protein product
5XTB_C	169.452	Chain C, NADH dehydrogenase [ubiquinone] iron-sulfur protein 7, mitochondrial

Top human protein match results comparing with the vesicular stomatitis virus proteome

Immune response against immunoglobulin heavy chain can cause immune complexes and result in diseases such as Kawasaki disease (24) and atherosclerosis.

Accession number	BLASTP score	Antigen
6RMG_A	751.813	Chain A, Protein patched homolog 1,GFP-like fluorescent chromoprotein FP506, related
CAH18708	39.6615	hypothetical protein, partial
AAH48327	36.2683	DIP2C protein, partial
AAM28524	35.42	tight junction protein 2
MOK23821	34.9959	immunoglobulin heavy chain junction region, partial
AHZ09053	33.7234	immunoglobulin heavy chain variable region, partial

Top human protein match results comparing with the rice proteome

Immune responses directed against these proteins can be expected to cause autoimmune retinopathy, autoimmune myelodysplastic syndromes, DNA repair related cancer, autoimmune macular degeneration, etc.

Accession number	BLASTP score	Antigen
NP_054733	3090.59	U5 small nuclear ribonucleoprotein 200 kDa helicase
NP_036565	2251.62	splicing factor 3B subunit 1 isoform 1
XP_011533696	2185.45	activating signal cointegrator 1 complex subunit 3 isoform X1
NP_000929	2177.39	DNA-directed RNA polymerase II subunit RPB2 isoform 1
NP_001309150	1911.87	ATP-dependent RNA helicase DHX8 isoform 8

Top human protein match results comparing with the Zaire ebolavirus envelope glycoprotein

immunoglobulin heavy chain variable region, partial [Homo sapiens]

[QGT40017.1](#) 119 1

Alignment statistics for match #1

Score	Expect	Identities	Positives	Gaps
34.1 bits(73)	3.8	11/21(52%)	12/21(57%)	9/21(42%)
Query	640	DNDNWWTGW-RQ-----WI	652	
		+NDNWWT W RQ WI		
Sbjct	30	NNDNWWT-WVRQPPGKGLEWI	49	

Diseases predicted based on homology of rice and African green monkey peptides to human autoimmune disease related epitopes in the IEDB

A single amino acid residue difference between a peptide in the vaccine and a self peptide results in the highest probability of vaccine induced autoimmune diseases. Two amino acid residue difference results in the next highest level probability of vaccine induced autoimmune diseases.

<u>Single amino acid difference</u>	<u>Two amino acid difference</u>
acquired epidermolysis bullosa	acquired epidermolysis bullosa
amyotrophic lateral sclerosis	alopecia areata
ankylosing spondylitis	amyotrophic lateral sclerosis
autoimmune atherosclerosis	ankylosing spondylitis
autoimmune glomerulonephritis	atherosclerosis
autoimmune hepatitis	autoimmune glomerulonephritis
autoimmune neuropathy	autoimmune haemolytic anaemia
autoimmune pancreatitis	autoimmune hepatitis
autoimmune polyendocrine syndrome	autoimmune neuropathy
autoimmune thyroiditis	autoimmune optic neuritis
autoimmune uveitis	autoimmune polyendocrine syndrome
autoimmune vasculitis	autoimmune thyroiditis
bullous pemphigoid	autoimmune vasculitis
celiac disease	bullous pemphigoid
chronic fatigue syndrome	celiac disease
cicatricial pemphigoid	chronic fatigue syndrome
Crohn's disease	cryoglobulinemia
cryoglobulinemia	demyelinating polyneuropathy
Goodpasture's syndrome	drug-induced lupus erythematosus
Grave's disease	Goodpasture's syndrome

<u>Single amino acid difference</u>	<u>Two amino acid difference</u>
Hirata disease	Grave's disease
insulin-dependent diabetes mellitus	Guillain-Barre syndrome
juvenile ankylosing spondylitis	Hirata disease
juvenile rheumatoid arthritis	insulin-dependent diabetes mellitus
lichen planus	juvenile ankylosing spondylitis
multiple sclerosis	juvenile rheumatoid arthritis
myasthenia gravis	Lambert-Eaton myasthenic syndrome
narcolepsy	multiple sclerosis
neuromyelitis optica	myasthenia gravis
non-insulin-dependent diabetes mellitus	narcolepsy
Parkinson's disease	neuromyelitis optica
pemphigus	non-insulin-dependent diabetes mellitus
pemphigus gestationis	pemphigus
prediabetes syndrome	prediabetes syndrome
psoriasis	psoriasis
reactive arthritis	reactive arthritis
relapsing polychondritis	relapsing polychondritis
rheumatic myocarditis	rheumatic myocarditis
rheumatoid arthritis	rheumatoid arthritis
rheumatologic disorder	rheumatologic disorder
sarcoidosis	sarcoidosis
Sjogrens syndrome	sclerosing cholangitis
systemic lupus erythematosus	Sjogrens syndrome
systemic scleroderma	stiff-person syndrome
thrombocytopenia	systemic lupus erythematosus

<u>Single amino acid difference</u>	<u>Two amino acid difference</u>
ulcerative colitis	systemic scleroderma
vasculitis	thrombocytopenia
Vogt-Koyanagi-Harada	vasculitis
Wegener's granulomatosis	vitiligo
	Wegener's granulomatosis

Sample single amino acid residue difference alignment to an IEDB peptide

DEVQVVRGHY is an ankylosing spondylitis related peptide in IEDB

BAF27610.1 partial [Oryza sativa Japonica Group]

Query DEVQVVRGHY

DEVQVVRG Y

Sbjt DEVQVVRGSY

Sample two amino acid residue difference alignment to an IEDB peptide

AETVQTVRY is an ankylosing spondylitis related peptide in IEDB

ABA93646.1 Transposable element protein, putative, Transposase_28 [Oryza sativa Japonica Group]

Query AETVQTVRY

AETVQT Y

Sbjct AETVQTMWY

Human carbonic anhydrase II homology to rice protein

This result means high probability of vaccine induced hematologic malignancies such as acute myeloid leukemia and other cancers (25).

Human carbonic anhydrase 2 (P00918) vs.

alpha carbonic anhydrase 7 [Oryza sativa Japonica Group]

[XP_015648465.1](#) 275 1

Alignment statistics for match #1

Score	Expect	Method	Identities	Positives	Gaps
110 bits(274)	5e-28	Compositional matrix adjust.	83/253(33%)	112/253(44%)	34/253(13%)
Query 8		GKHNGPEHWHKDFP----	IAKGERQSPVDIDHTAKYDPSLKPLSVSYDQATSLRILNNG		63
		G GPEHW K P	GE QSP+D+ K L L SY +A I+N G		
Sbjct 41		GDEKGPEHWGKLPKPEWAQCGAGEMQSPIDLSHERVKLVRLDGLYLDSDY-RAAEASIVNRG			99
Query 64		HAFNVEFDDSDQKAVLKGGLDGTyrLIQFHFHWGSLDGQGSEHTVDKKKYAAELHLVHW			123
		H V FD V+ G Y L Q H+H +EH+VD ++Y ELH+VH			
Sbjct 100		HDIMVRFDGDAGSVVINGT-----AYYLRQLHWH-----SPTESVDGRRYDMELHMHVHE			149
Query 124		NTKYGDFGKAVQQPDGLAVLGIFLKVGSAPGLQKVVDVLDSIKTK-GKSADFTNFDPRG			182
		+ + AV+G+ +VG LQK+ L I K + DPRG			
Sbjct 150		SAE-----KKAIVIGLLYEVRPDRFLQKMEPYLKMIADKEDREEKVG MIDPRG			198
Query 183		LLPESLDYWTYPGSLTTPPLLECVTWIVLKEPISVSSEQVLKFRKLNFNNGEGEPEELMVD			242
		+ Y+ Y GSLTTPP + V W ++K +VS Q+ R+ + M +			
Sbjct 199		ARGRASVYYRYMGSLTTPPCTQGVVWTIVKRVRTVSRYQLDLLREAVHDE-----MEN			251
Query 243		NWRPAQPLKNRQI	255		
		N RP Q + NR I			
Sbjct 252		NARPLQAVNNRDI	264		

Discussion

As described previously, a single amino acid residue difference between a self peptide and antigen peptide is ideally suited to invoke an autoimmune response (26). Animal and plant proteins have numerous locations where the peptides differ by one or more amino acid residues compared to homologous human self-peptides. These are ideally suited to cause low affinity self reactive (LASR) T cell mediated autoimmunity as previously described (26).

The dengue vaccine - Dengvaxia, failed because of waning immunity following the vaccine, coupled with vaccine induced long term persistent IgE directed at the viral proteins in the vaccine (27). The result is, once the vaccine induced immunity wanes, the secondary infection is severe due to an allergic reaction occurring concurrently with the infection. The same occurs with the influenza vaccine (28). We can expect the exact same result with ERVEBO. Once ERVEBO induced immunity wanes, an Ebola infection can be expected to be even more severe due to a concurrent IgE mediated hypersensitivity reaction directed against the Zaire ebolavirus envelope glycoprotein.

Conclusion

This vaccine is only useful for limited administration in “ring vaccination” strategies to contain an Ebola outbreak. This vaccine is unacceptable and unsafe for prophylaxis in the general population using mass vaccination. It will cause epidemics of rice allergy, numerous autoimmune diseases and cancer.

Fundamental redesign is needed to alter the route of administration and/or removal of ALL non-target antigens to improve vaccine safety.

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