Analyzing 23000+ epitopes covering 82 autoimmune diseases in the Immune Epitope Database, 57% have only one and 78% have up to two amino acid residue differences compared to animal, fungal or plant peptides present in vaccines; an unmistakable signature of the role of vaccines in their etiologies

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Abstract

The National Institute of Allergy and Infectious Diseases (NIAID) sponsors the Immune Epitope Data Base (IEDB). IEDB contains epitopes identified from the medical literature and organized by diseases and categories of diseases. All epitopes (23000+) associated with 82 autoimmune diseases in humans were analyzed.
The role of animal, plant, fungal proteins contained in vaccines in the etiology of autoimmune diseases have been described in humans and animals. BLASTP was used to analyze IEDB derived epitopes for sequence alignment to animal, plant, fungal (APF) proteins present in vaccines and biologics. Specifically, the search was performed against bovine, chicken, porcine, guinea pig, African green monkey, Chinese hamster, murine, peanut, soy, wheat, corn, sesame and *Saccharomyces cerevisiae* proteomes.

The results show that 57% of epitopes differed by exactly one amino acid residue from an APF peptide. 78% of the epitopes differed by up to two amino acid residues from an APF peptide. The rest of the epitopes were either identical or differed by more than two amino acid residues.

A majority of IEDB epitopes analyzed were 9-mer peptides. Comparing randomly selected 9-mer human peptides with APF proteomes, the probability of single amino acid residue difference (SAARD) outcomes was derived. This was used to estimate the probability that actual IEDB SAARD alignments to APF peptides were merely a chance outcome. The estimates show that the probability that the observed IEDB alignments to APF being merely a chance outcome are vanishingly small. So the results make it absolutely clear that APF proteins in vaccines cause all these autoimmune diseases.

Introduction

Vaccines contain thousands of residual animal, plant and fungal (APF) proteins from the manufacturing process (1–6). 293 chicken proteins were identified in the influenza vaccine (7), for example. Actin and vimentin proteins were detected in the Priorix Tetra vaccine (8). Immunization with homologous xenogeneic antigens resulting in autoimmune diseases has been known for at least 40 years (9). Vaccines that contain bovine proteins caused

autoimmunity in dogs (10). We previously described the immunological mechanism involved in autoimmunity induction by immunization with homologous xenogeneic antigens (11).

Cancer cells have minor differences when compared to healthy cells. Due to mutation of the DNA encoding the proteins, cancer cells can display altered proteins on their surface. Healthy cancer defense mechanisms include immune responses directed at such altered proteins. Therefore an immune response directed against cancer cells always carries a risk of cross reactive immune responses against healthy cells displaying the unaltered protein. Therefore, cancer induced autoimmune responses are a consequence of normal, healthy immune system behavior.

Animal proteins have minor differences compared to human proteins. Peptides that are identical between humans and animals are unlikely to cause any problem due to strong self tolerance. However, peptides with one amino acid residue difference produce the strongest cross reactive immune responses (11). They are ideally suited to induce autoimmune diseases. Injecting animal proteins results in anti-cancer immune responses because the immune system perceives animal proteins as altered human proteins. Adjuvants in the vaccine boost this anti-cancer immune response. This artificial anti-cancer response directed at thousands of APF proteins in vaccines or biologics, therefore cross react and cause numerous autoimmune disorders.

For this reason, one can predict that single amino acid residue difference (SAARD) between autoimmune disease related epitopes in the IEDB and homologous APF peptides in vaccines, would occur at a higher probability than by mere chance. We perform a BLASTP analysis to verify.

Methods

Basic local alignment search tool for proteins (BLASTP) (12), Universal Protein Resource (UniProt)(13) and the Immune Epitope Database (IEDB) (14) were used for bioinformatics analysis.

Specifically, the BLASTP sequence alignment of IEDB peptides was performed against bovine, chick, porcine, guinea pig, African green monkey, Chinese hamster, murine, peanut, soy, wheat, corn, sesame and *Saccharomyces cerevisiae* proteomes. Vaccines and biologics contain residual proteins from all these organism due to media used to grow viruses or bacteria, recombinant cells/organisms used for protein expression or as excipients.

Results

57% of IEDB peptides have a SAARD and 78% have up to two amino acid differences compared to animal, fungal or plant peptides present in vaccines.

Discussion

<u>Could this result be a chance occurrence?</u>

A majority of IEDB epitopes analyzed were 9-mer peptides. Five thousand 9-mer peptides were chosen at random from the human proteome. BLASTP was run using these peptides to compare against each organism's proteome or a subset. This provides us the probability that randomly selected human peptides have an alignment providing a SAARD compared to peptides from these organisms. These are listed in table 1 under " Random SAARD alignment". Given this, we can compute the probability of the actual SAARD alignment to IEDB epitopes, occurring by chance. This is listed in table 1 under "Estimated probability of actual SAARD outcome occurring just by chance".

For a simple coin toss example, we would perform the calculation as follows:

Computing probability of say a 7 heads, 3 tails outcome of 10 trials of a fair coin:

Probability = (0.5^7) x (0.5^3) x 10! / (7! x 3!)
Where:
0.5 is the probability of a head or tail outcome of a fair coin.
For an unfair coin, say probability of head outcome = 0.4 and tail outcome 0.6, we would have:
Probability = (0.4^7) x (0.6^3) x 10! / (7! x 3!)

For the IEDB peptide probability analysis, the outcome is for 23192 trials (peptides).
The "head outcome" is the "Random SAARD alignment " entries in table 1. The "tail outcome" probability is 1-"head outcome".

Sample calculation for Chinese Hamster:

Probability = ((889/5000)^4574) x ((4111/5000)^18618) x 23192! / (4574! x 18618!)

Probability = 1.488e-15

Where: 889*100/5000 = 17.8% is the entry in Table 1 for Chinese Hamster. BLASTP analysis shows 889 SAARD out of 5000 peptides analyzed.
And, 4574*100/23192=19.7% is the IEDB entry in Table 1 for Chinese Hamster. BLASTP analysis shows 4574 SAARD out of 23192 peptides analyzed.

So the 1.488e-15 value is the probability that we will have exactly 4574 SAARD alignments out of 23192 peptides. The probability goes down for all values > 4574. Conservatively, applying the same probability as for 4574, to all values >4574, we can calculate the probability of the chance occurrence of 4574 or greater number of SAARD alignments as 1.488e15 * 18618 = 27e-12, entry in table.

The Gnome calculator was used to perform these calculations and the results were verified using the Qalculate! calculator and WolframAlpha (15) since spreadsheets are unable to perform these calculations.

Table 1

Organism	Random SAARD alignment Number of Peptides(%)	Actual (IEDB) SAARD alignment Number of Peptides (%)	Estimated probability of actual SAARD outcome occurring just by chance	Remarks
African green monkey (*Chlorocebus aethiops*)	26 (0.5)	420 (1.8)	~1.7e-96	Probability of this outcome occurring just by chance is vanishingly small. So these animal proteins in vaccines, caused these diseases.
Cow (*Bos taurus*)	936 (18.7)	4385 (18.9)	~1	There are two possibilities. (1) This outcome occurred just by chance. (2) The assumption that all cow proteins are present in the vaccines in equal amount is not true. We know that cow's milk, bovine gelatin, bovine serum albumin are used in vaccines. So these proteins and proteins present in tissues in the vicinity, are included in vaccines but other cow proteins may not be present. This is the more likely explanation. Please see Cow's milk entry below.
Cow's Milk (*Bos taurus*)	0 (0)	12 (0.05)	0	Probability of this outcome occurring just by chance is 0. So these animal proteins in vaccines, caused these diseases.
Chinese Hamster (*Cricetulus griseus*)	889 (17.8)	4574 (19.7)	~27e-12	Probability of this outcome occurring just by chance is vanishingly small. So these animal proteins in vaccines, biologics caused these diseases.
Chicken (*Gallus gallus*)	536 (10.7)	3901 (16.8)	<1e-100	Probability of this outcome occurring just by chance is vanishingly small. So these animal proteins in vaccines, caused these diseases. Unlike bovine, porcine and murine proteins, since chicken egg or embryo is used for vaccine manufacture, all chicken proteins can be present in vaccines.
Guinea Pig (*Cavia porcellus*)	837 (16.7)	4439 (19.1)	~1e-18	Probability of this outcome occurring just by chance is vanishingly small. So these animal proteins in vaccines, caused these diseases.
Mice (*Mus musculus*)	940 (18.8)	4467 (19.3)	~1	There are two possibilities. (1) This outcome occurred just by

Organism	Random SAARD alignment Number of Peptides(%)	Actual (IEDB) SAARD alignment Number of Peptides (%)	Estimated probability of actual SAARD outcome occurring just by chance	Remarks
				chance. (2) The assumption that all mice proteins are present in biologics in equal amount is not true. We know that mice myeloma cells are used in biologics. So these proteins and proteins present in tissues in the vicinity, are present in biologics but other mice proteins may not be present. This is the more likely explanation.
Maize (*Zea mays*)	303 (6.1)	1667 (7.2)	~5e-9	Probability of this outcome occurring just by chance is vanishingly small. So these plant proteins in vaccines, caused these diseases.
Peanut (*Arachis hypogaea)*	223 (4.5)	1697 (7.3)	~79e-81	Probability of this outcome occurring just by chance is vanishingly small. So these plant proteins in vaccines, caused these diseases.
Yeast *(Saccharomyces cerevisiae)*	64 (1.3)	828 (3.6)	<1e-100	Probability of this outcome occurring just by chance is vanishingly small. So these fungal proteins in vaccines, biologics caused these diseases.
Sesame (*Sesamum indicum*)	155 (3.1)	1398 (6.0)	<1e-100	Probability of this outcome occurring just by chance is vanishingly small. So these plant proteins in vaccines, caused these diseases.
Soy (*Glycine max)*	215 (4.3)	1548 (6.7)	~86e-59	Probability of this outcome occurring just by chance is vanishingly small. So these plant proteins in vaccines, caused these diseases.
Pig (*Sus scrofa*)	963 (19.3)	4407 (19.0)	~1	There are two possibilities. (1) This outcome occurred just by chance. (2) The assumption that all porcine proteins are present in the vaccines in equal amount is not true. We know that porcine gelatin is used in vaccines. So these proteins and proteins present in tissues in the vicinity are included in vaccines but other porcine proteins may not be

Organism	Random SAARD alignment Number of Peptides(%)	Actual (IEDB) SAARD alignment Number of Peptides (%)	Estimated probability of actual SAARD outcome occurring just by chance	Remarks
				present. This is the more likely explanation.
Wheat (*Triticum aestivum*)	138 (2.8)	977 (4.2)	~18e-33	Probability of this outcome occurring just by chance is vanishingly small. So these plant proteins in vaccines, caused these diseases.

The calculations make it clear that the findings cannot be merely a chance outcome and that immunization against animal/plant/fungal antigens in the vaccines do cause these autoimmune diseases in the IEDB.

Conclusion

Vaccines containing animal, plant or fungal proteins are extremely dangerous and cause numerous autoimmune diseases and cancer (16–19). All non-target proteins in vaccines must be immediately removed using processes such as affinity chromatography (20).

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