Immunogenicity Analysis of Chinese Hamster Ovary (CHO) Host Cell Protein Contaminants in Therapeutic Protein Formulations

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Abstract

Concerns over immune responses to contaminating host cell proteins (HCPs) in therapeutics have recently emerged. For example, the presence of Chinese hamster ovary (CHO)-derived HCPs contributed to the cancellation of two phase III clinical trials in 2012 for Inspiration's IB1001, a recombinant factor IX produced in CHO cells. These trials were cancelled due to the development of anti-CHO antibodies at higher levels than expected in patients treated with a protein drug. Publication of the CHO-K1 genome and transcriptome offers an opportunity to analyze and understand the immunogenic potential of CHO HCPs.

We analyzed the CHO-K1 genome, the CHO-K1 transcriptome, published CHO proteins identified in biotherapeutics, and mouse secreted proteins downloaded from the LOCATE and UniProtKB/Swiss-Prot databases. These datasets were screened for potential $CD4^+$ T cell immunogenicity using the epitope-mapping algorithm EpiMatrix and overall protein immunogenicity scores calculated. Human homologs discovered by BLAST were similarly screened for T-cell epitope content; predicted immunogenicity was then adjusted for human homology.

Further study is required to assess the potential for autoimmune responses, as several widely publicized cases of severe adverse immune responses to autologous therapeutic proteins are published and the FDA and EMA take the potential for such effects very seriously. Finally, we built a website (CHOPPI) to give protein manufacturers free access to information on potential immunogenicity of CHO-K1 sequences. We look forward to its further development in collaboration with protein therapeutics industry, as this and additional means of performing immunogenicity analysis continue to be refined.

CHO Cell Protein

- CHO-derived Host Cell Protein (HCP) contaminants in biotherapeutics can potentially generate immune responses.
- Of significant concern are immune responses that may be cross-reactive with human T cell epitopes.

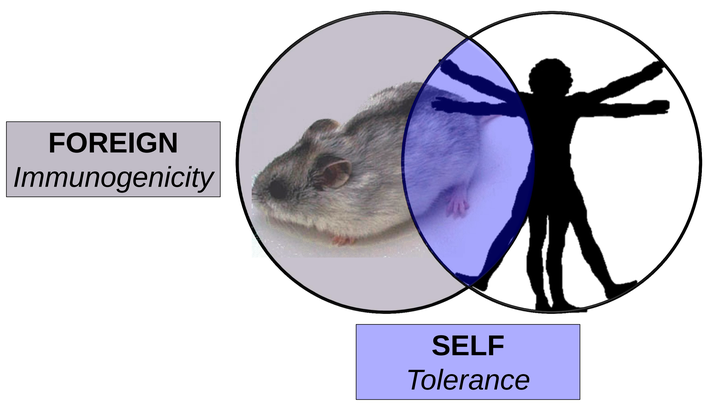


Figure 1. Immune response to mammalian proteins.

- The availability of the CHO genome and transcriptome has made it possible to apply immunoinformatics tools to HCP analysis.
- This significantly accelerates research on HCP immunogenicity and cross-reactivity.

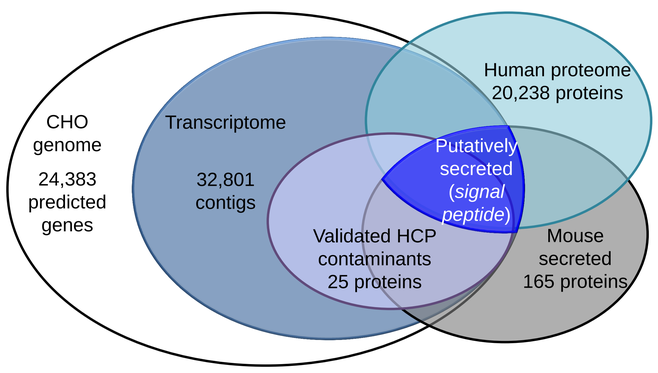
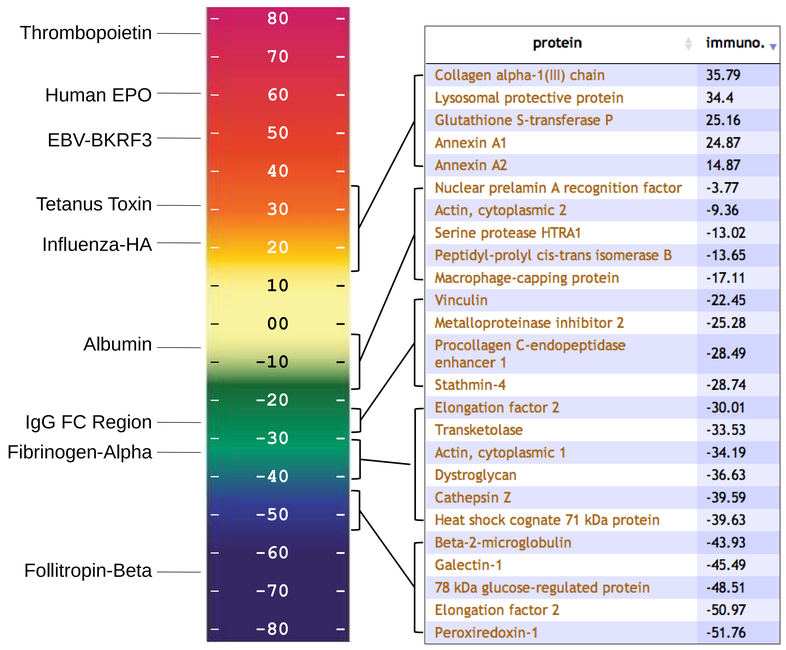


Figure 2. Databases used for immunogenicity and cross-reactivity analysis. *Illustration of the sources used to analyzed potential CHO HCP immunogenicity Sources include CHO genome, CHO transcriptome, published CHO proteins identified in biotherapeutic products (Experimental HCP contaminants), and mouse secreted proteins downloaded from LOCATE and UniProtKB/Swiss-Prot.*

Immunogenicity Analysis

Immunogenic potential of validated HCP contaminants was assessed by epitope density and ranked on an immunogenicity scale, which was developed to evaluate therapeutic proteins of interest against known immunogenic and non-immunogenic proteins.



protein	immuno.
Collagen alpha-1(III) chain	35.79
Lysosomal protective protein	34.4
Glutathione S-transferase P	25.16
Annexin A1	24.87
Annexin A2	14.87
Nuclear prelamin A recognition factor	-3.77
Actin, cytoplasmic 2	-9.36
Serine protease HTRA1	-13.02
Peptidyl-prolyl cis-trans isomerase B	-13.65
Macrophage-capping protein	-17.11
Vinculin	-22.45
Metalloproteinase inhibitor 2	-25.28
Procollagen C-endopeptidase enhancer 1	-28.49
Stathmin-4	-28.74
Elongation factor 2	-30.01
Transketolase	-33.53
Actin, cytoplasmic 1	-34.19
Dystroglycan	-36.63
Cathepsin Z	-39.59
Heat shock cognate 71 kDa protein	-39.63
Beta-2-microglobulin	-43.93
Galectin-1	-45.49
78 kDa glucose-regulated protein	-48.51
Elongation factor 2	-50.97
Peroxiredoxin-1	-51.76

Figure 3. EpiMatrix Protein Immunogenicity Scale. *Validated CHO HCP contaminants with scores ≥20 represent approximately the top 20% of the distribution. High-scoring proteins are considered potentially immunogenic. Conversely, proteins scoring below -20 are categorized as possessing "low" immunogenic potential. All scores normalized for protein length*

Immunogenicity comparison

- Immunogenicity scores calculated for proteins sourced from three different datasets.
- High-scoring proteins (above 20) are considered potentially immunogenic.
- Proteins scoring between -20 and 20 are less likely to generate a significant immune reaction.
- Major validated HCP contaminants peak co-locates with secreted proteins peak, suggesting lower potential immunogenicity for this set,
- A minor peak higher on the scale suggests that some HCP do carry a risk of immunogenicity.

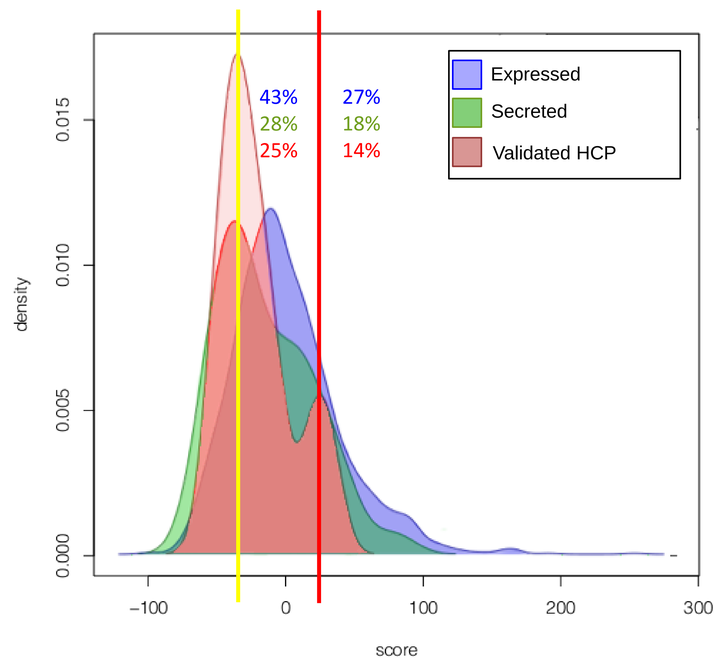


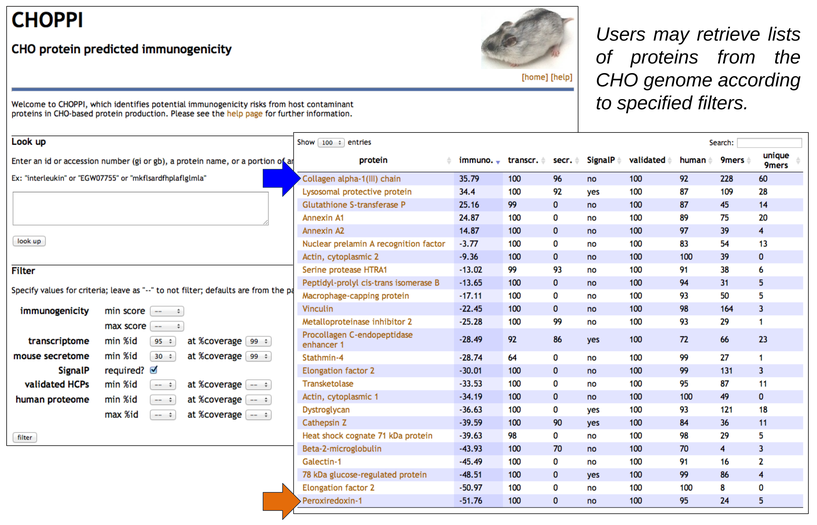
Figure 4. Kernel density estimate of potential and validated CHO HCP immunogenicity scores. *Shoulders in validated HCP contaminants (red) and putative secreted protein (green) sets arise due to higher subpopulation scores. Area under each curve is 1. Lines delimitate immunogenicity potential (yellow: neutral; red: high). Percentages are % predicted proteins with reduced (yellow to red lines) or increased (red line rightward) immunogenicity potential.*

CHOPPI Web Tool

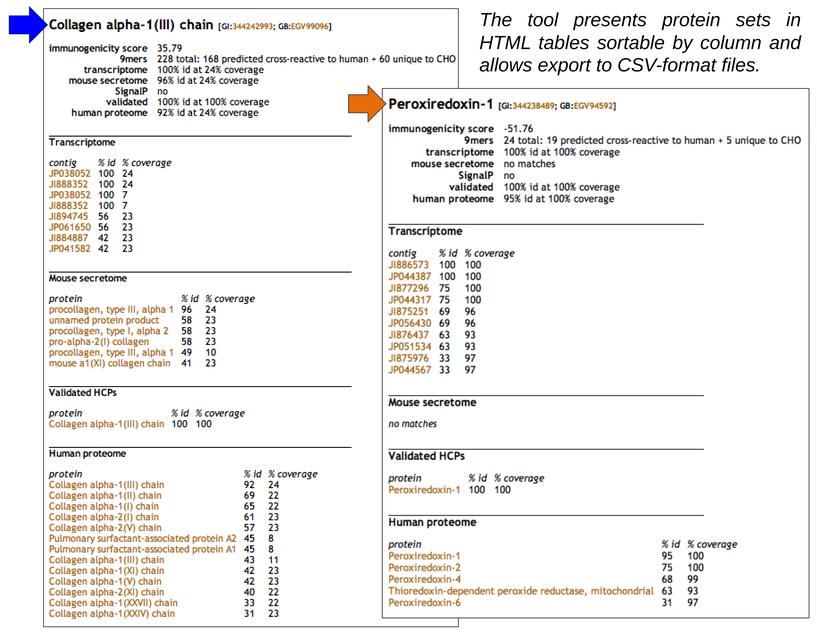
CHOPPI (CHO Protein Predicted Immunogenicity) is a web tool developed to address two questions:

(1) **What are the possible contaminants?** CHOPPI integrates the genome of the CHO-K1 cell line, a CHO transcriptome, a mouse secretome, and SignalP-based signal peptide prediction (indicating likely secretion).

(2) **Which are likely to be immunogenic?** CHOPPI assesses predicted T cell epitope content by EpiMatrix, as well as compares the proteins to their human homologs and their predicted epitopes to predicted epitopes within the human genome.



Users may retrieve lists of proteins from the CHO genome according to specified filters.



The tool presents protein sets in HTML tables sortable by column and allows export to CSV-format files.

Figure 5. CHOPPI web tool (http://clover.cs.dartmouth.edu/~cbk/choppi). *Each protein is characterized in terms of homology criteria and signal peptide prediction, along with assessments of epitope count and density, and count and density of epitopes unique to CHO according to a human cross-reactivity analysis.*

Conclusions

- The presence of HCP in therapeutic protein products may contribute to adverse clinical side effects and may impact drug efficacy.
- The impact of cross-conserved epitopes on human immune responses may include induction of effector T cells, induction of Tregs, or anergy.
- Our ability to score HCP for immunogenicity opens the door for future analysis such as the screening of whole proteins from the CHO transcriptome.
- Future HCP immunogenicity scores will be adjusted based on the prevalence of the human homolog in human extracellular fluids (such as serum).
- In vitro studies will be required to determine the impact of our findings and their implications.

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