Lack of evidence of significant homology of SARS-CoV-2 spike sequences to myocarditis-associated antigens

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Background and potential hypotheses

- COVID-19 mRNA vaccines have proven to be highly safe and effective
- Myocarditis is a rare adverse event associated with mRNA vaccination, especially in young males
- Various hypotheses have been set forth to explain these event, including alterations in innate immunity, detrimental SARS CoV2 adaptive immune responses and autoimmunity
- As myocarditis can be driven by autoimmune responses, we examined if SARS-CoV-2 spike was potentially cross-reactive with autoantigens previously associated with myocarditis
  - Sequence identity comparison between SARS-CoV-2 spike protein-derived peptides and myocarditis-associated antigens
  - Structural analysis of these antigens and SARS-CoV-2 spike to identify potential discontinuous 3-D epitope similarities

Protein Name	Gene	UniProt ID	Source
Myosin-6	MYO6	Q9UM54	IEDB
Myosin-7	MYH7	P12883	IEDB
Muscarinic acetylcholine receptor M2	CHRM2	P08172	IEDB
Myosin-binding protein C - cardiac-type	MYBPC3	Q14896	IEDB
Myosin-binding protein C - fast-type	MYBPC2	Q14324	IEDB
Beta-2-glycoprotein 1	APOH	P02749	IEDB
Laminin subunit alpha-1	LAMA1	P25391	IEDB
Transmembrane protease serine 4	TMPRSS4	Q9NRS4	IEDB
Troponin I	TNNI3	P19429	Review Literature
Troponin T	TNNT2	P45379	Review Literature
Beta-1 adrenergic receptor	ADRB1	P08588	Review Literature
Actin, alpha cardiac muscle 1	ACTC1	P68032	Review Literature
Tropomyosin alpha-1 chain	TPM1	P09493	Review Literature
Tropomyosin beta chain	TPM2	P07951	Review Literature
Tropomyosin alpha-3 chain	TPM3	P06753	Review Literature
Cytoplasmic aconitate hydratase	ACO1	P21399	Review Literature
ADP/ATP translocase 1	SLC25A4	P12235	Review Literature
Creatine kinase B-type	CKB	P12277	Review Literature
Creatine kinase S-type, mitochondrial	CKMT2	P17540	Review Literature
Creatine kinase U-type, mitochondrial	CKMT1A	P12532	Review Literature
Creatine kinase M-type	CKM	P06732	Review Literature
Desmin	DES	P17661	Review Literature
Dihydrolipoyl dehydrogenase, mitochondrial	DLD	P09622	Review Literature
60 kDa heat shock protein, mitochondrial	HSPD1	P10809	Review Literature
Heat shock 70 kDa protein 1A	HSPA1A	P0DMV8	Review Literature
Vimentin	VIM	P08670	Review Literature
E3 ubiquitin-protein ligase TRIM21	TRIM21	P19474	Review Literature
Lupus La protein	SSB	P05455	Review Literature
Pyruvate kinase	PKLR	P30613	Review Literature
Ubiquinol-cytochrome-c reductase complex assembly factor 1	UQCC1	Q9NVA1	Review Literature
Sodium/potassium-transporting ATPase subunit alpha-1	ATP1A1	P05023	Review Literature
Natriuretic peptides B	NPPB	P16860	Review Literature
Natriuretic peptides A	NPPA	P01160	Review Literature
Troponin C, slow skeletal and cardiac muscles	TNNC1	P63316	Review Literature
Transmembrane protein 65	TMEM65	Q6PI78	Review Literature

***Table 1*: Myocarditis-Associated Cardiac Antigens.**

A set of approximately 40 different antigens was considered

Homology of 9 mers and 15 mers (representative of sizes recognized by CD8 and CD4 T cells respectively)

	Match in Cardiac Proteins	No Match in Cardiac Proteins	Total Peptides
Spike Peptides	13	1246	1259
Shuffled Peptides	14	1245	1259
Total Peptides	27	2491	2518

***Table 2*: SARS-CoV-2 Spike 15-mers vs. Shuffled 15-mers (Homology >= 53%).**

	Match in Cardiac Proteins	No Match in Cardiac Proteins	Total Peptides
Spike Peptides	77	1188	1265
Shuffled Peptides	55	1210	1265
Total Peptides	132	2398	2530

***Table 3*: SARS-CoV-2 Spike 9-mers vs. Shuffled 9-mers (Homology >= 67%).**

- While some hits were observed using very loose homology criteria, no difference was observed between spike and “shuffled peptides “ control
- This was true for both peptide lengths

Similar results were observed when the homology to potential myorcaditis autoantigens was compared to the rest of the genome

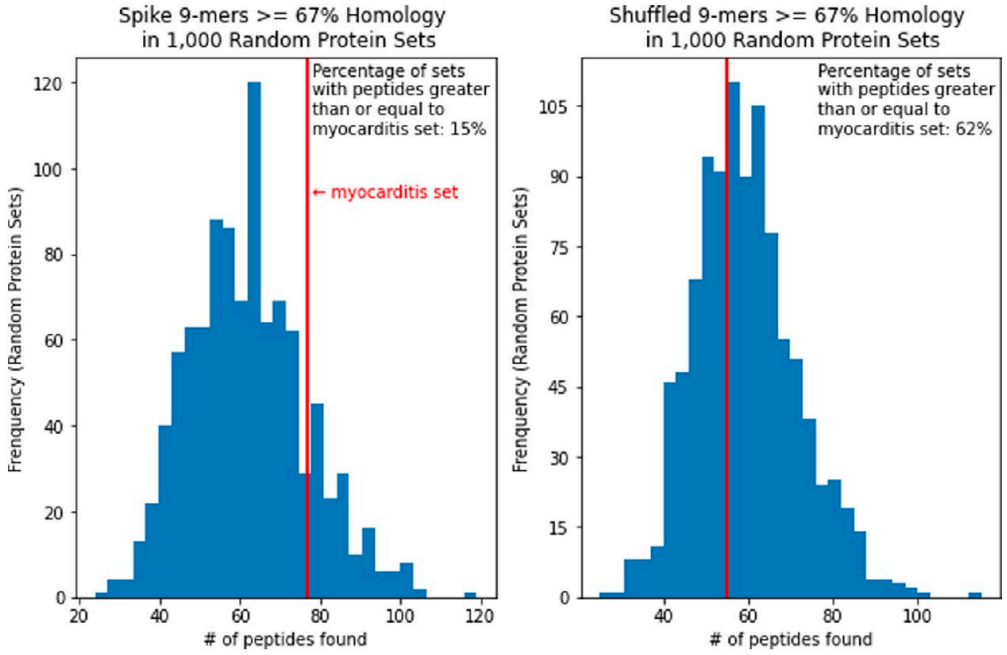

Figure 1. Spike vs shuffled 9-mers >= 67% homology match distribution of 1000 random protein sets.

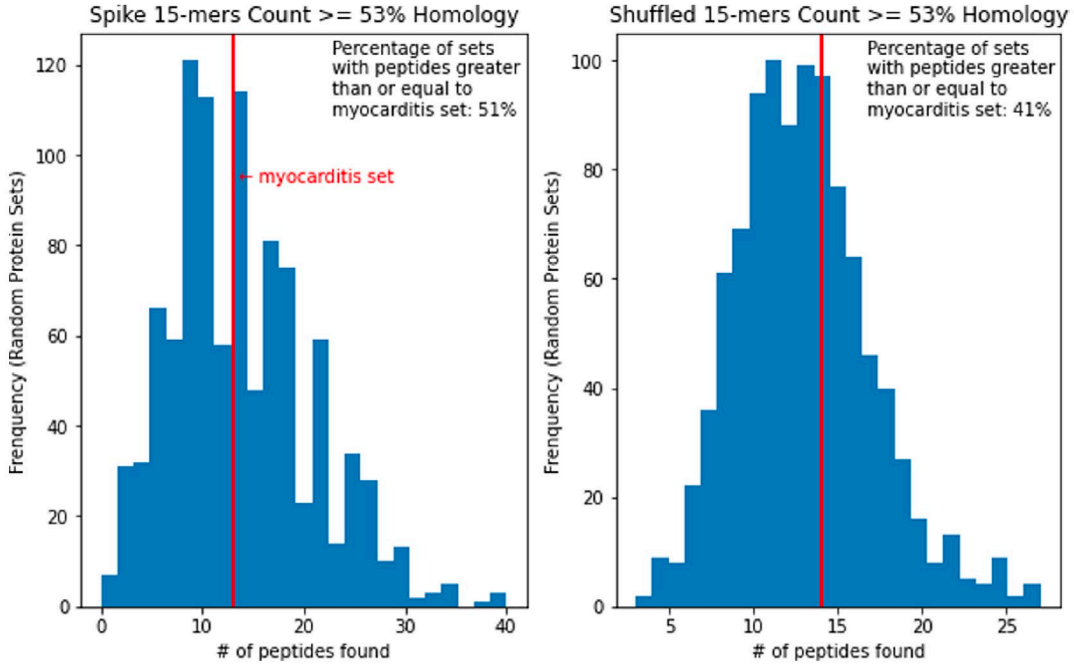

Figure 2. Spike vs shuffled 15-mers >= 53% homology match distribution of 1000 random protein sets.

3-D structural analysis to probe for homology of potential relevance for antibody recognition

PDB ID	Source Protein	UniProt ID	TM-align Score	Residue count
3SSU	Vimentin	P08670	0.742	91
5KHT	Tropomyosin alpha-1 chain	P09493	0.857	29
5WLQ	Myosin-7	P12883	0.516	79
6OTN	Tropomyosin alpha-3 chain	P06753	0.621	74

***Table 4*: Significant TM-align scores for antigen fragments compared with spike.**

- PDB files for myocarditis-associated antigens were compared to the SARS-Cov-2 Spike protein using the TM-align program with the structure of the spike protein
- TM-align scores are considered significant when greater than or equal to 0.5.
- Four substructures of these antigens had significant scores
- These residues are considered to have a low solvent-accessible surface area
- These antigens were not significantly higher in structural similarity compared with controls

Additional considerations

- Our study does not provide evidence for cross-reactive recognition, but is limited by the accuracy of the computational approach and experimental analysis has not been performed
  - Lack of evidence is not evidence of lacking
- The lack of reported adaptive autoimmunity is consistent with the self-resolving nature of the events
- Direct immediate causation from innate immunity is not immediately consistent with a delayed onset
  - Autoantibodies to IL1RA could mediate hyperinflammation
  - Yonker et al point to unbound circulating Spike in myocarditis subjects, not inducing immune hyperactivation, but potential direct effects
- Myocarditis as an adverse event was also observed in the case of other vaccines (smallpox)
  - Compatible with an IFN-gamma/ inflammatory reaction caused by the adaptive response, secondary to a rare pre-existing condition of vulnerability of the heart tissue
- Additional experimental studies to address these issues
  - Difficulties in obtaining pre-post adverse event samples
  - Possibilities include GWAS, HLA and genetic studies, and adaptive memory responses studies

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