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Anti-SARS-CoV-2 Immune Response and Sudden Death: Titin as a Link

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Abstract

Twenty-nine pentapeptides are common to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) spike glycoprotein (gp) and the cardiac human protein Titin, alterations of which are linked to cardiac failure and sudden death. The peptide commonality does not reflect mere peptide sharing, rather it has relevant immunological potential as nearly all of the shared sequences are present in experimentally validated SARS-CoV-2 spike gp-derived epitopes, thus supporting the possibility that anti-Titin autoimmune cross-reactions can be triggered by the virus. Moreover, contextual peptide commonality among SARS-CoV-2, human Titin, and microbial organisms to which individuals have previously been exposed, highlights the likelihood of anamnestic and extremely powerful anti-Titin immune responses that may derive from earlier immunologic imprinting. The present data may help understand, explain, and diagnose the relationship between sudden death and infections.

Keywords: SARS-CoV-2; Titin; peptide sharing; cross-reactivity; immunologic imprinting; sudden death; post-mortem autopsy; sudden death diagnosis

1 Introduction

Evidence suggests that autoimmunity may contribute to sudden death [1-5]. However, the molecular basis and the mechanism that trigger autoimmune reactions disruptive of the cardiac function are unknown. Recently, the issue assumed a particular importance in light of the increased incidence of out-of-hospital sudden death that has been reported during the SARS-CoV-2 pandemic [6-9].

Hence, to explore the molecular basis that might link SARS-CoV-2 infection and sudden death, this study analyzed the viral spike gp for amino acid (aa) sequence identity to the cardiac protein Titin that – when mutated or altered – can associate with

myopathy and early respiratory failure [10]; hypertrophic cardiomyopathy characterized by ventricular hypertrophy with symptoms that include dyspnea, syncope, collapse, palpitations, and chest pain, and that are readily provoked by exercise [11]; dilated cardiomyopathy with ventricular dilation and impaired systolic function, resulting in arrhythmia and risk of premature death [12]; and sudden death [13, 14].

Mechanistically, the rationale behind the present research is that, given the massive peptide sharing between microbial and human proteomes [15, 16], infections may evoke immune responses that cross-react with human proteins, thus causing harmful autoimmunity [17-19].

2 Methods

Peptide matching was conducted on the spike gp (NCBI, GenBank Protein Accession Id=QHD43416.1) from SARS-CoV-2 (NCBI:txid2697049) and the human Titin protein (UniProt accession: Q8WZ42; www.uniprot.org/uniprot/Q8WZ42) [20]. Pentapeptides were used as sequence probes since five aa residues define a minimal immune determinant that can 1) induce specific antibodies, and 2) determine antigen-antibody specific interactions [21, 22].

The spike gp primary sequence was dissected into pentapeptides offset by one residue (that is: MFVFL, FVFLV, VFLVL, FLVLL and so forth) and the resulting viral pentapeptides were analyzed for occurrences within the Titin protein primary sequence. Pentapeptides common to spike gp and Titin were also analyzed for occurrences in *Bordetella pertussis* (NCBI:txid257313), *Clostridium tetani* (NCBI:txid212717), *Corynebacterium diphtheriae* (NCBI:txid257309), *Neisseria meningitidis* (NCBI:txid122586), and Poliovirus 1 (NCBI:txid12081) by using Uniprot resources and peptide search programs (www.uniprot.org/peptidesearch/; research.bioinformatics.udel.edu/) [20].

The immunological potential of the peptides shared between the spike gp and the human Titin was analyzed by searching the Immune Epitope DataBase (IEDB, www.iedb.org/) [23] for immunoreactive SARS-CoV-2 spike gp-derived epitopes hosting the shared pentaptides.

3 Results

Following sequence analyses, it was found that a high number of pentapeptides (namely, 29) are shared by SARS-CoV-2 spike gp and the cardiac Titin protein,. The shared pentapeptides are described in Table 1.

Table 1. Description of 29 pentapeptides common to the viral spike gp and Titin*

AENSV; AGAAA; ALDPL; DPLSE; EFRVY; FKEEL; GKLQD; ILDIT; INITR; **ISVTTE**; LGDIS; LQDVV; LSSTA; PKKST; PLVSS; PPAYT; **PSKPSK**; SEPVL; SLLIV; SNLKP; STNLV; TKTSV; **TLEILD**; TPPIK; VEAEV; VSMTK

*Hexapeptides derived from overlapping pentapeptides are given bold

The sharing reported in Table 1 represents a peptide platform for possible cross-

reactions between the virus and Titin. The hypothesis of a possible autoimmune cross-reactivity is supported by the fact that the peptide overlap between the viral gp and the cardiac protein has also a relevant immunological significance as shown in Table 2.

Table 2. Distribution of 29 pentapeptides shared between SARS-CoV-2 spike gp and Titin among 68 experimentally validated SARS-CoV-2 spike gp-derived epitopes

ID[1]	Epitope[2]	ID[1]	Epitope[2]
10112	dsFKEELdky	1309573	rlfrkSNLKPferdisteiy
57592	SEPVLkgvkl	1309585	sssgwtAGAAAyyvgylqpr
1072965	svtteilpVSMTKts	1309593	titdavdcALDPLSEtkctl
1074201	ylyrlfrkSNLKPfe	1309595	tnftISVTTEilpVSMTKts
1074866	cALDPLSEtk	1309598	tvydplqpeldsFKEELdky
1074872	dlpigINITRfqtl	1309602	vcgPKKSTnlvknkcvnfnf
1074876	eILDITpcsf	1309604	vlndilsrldkVEAEVqidr
1074898	ftISVTTEil	1309624	yyhknnkswmesEFRVYssa
1074928	ilpdPSKPSK	1310254	AENSVaysnnsiaip
1074961	kswmesEFRVY	1310336	dsktqSLLIVnnatn
1074969	lgAENSVaysnn	1310392	fgttldsktqSLLIV
1074980	lPPAYTnsf	1310448	GKLQDVVnqnaqaln
1074989	LSSTAsalgk	1310474	hknnkswmesEFRVY
1075117	wtAGAAAyyvgy	1310487	igINITRfqtllalh
1087408	teilpVSMTK	1310609	lpdPSKPSKrsfied
1087780	vkqiykTPPIKdfggfnf	1310614	lqpeldsFKEELdky
1309118	gPKKSTnlvknkcvn	1310803	siiaytmslgAENSV
1309132	nfsqilpdPSKPSKr	1310810	SLLIVnnatnvvikv
1309441	aysnnsiaiptnftISVTTE	1310850	TLEILDITpcsfggv
1309444	davrdpqTLEILDITpcsfg	1310852	Tlvkqlssnfgaiss
1309447	dfggfnfsqilpdPSKPSKr	1310855	tnftISVTTEilpvs
1309450	DPLSEtkctlksftvekgiy	1310899	vllPLVSSqcvnltt
1309451	dsFKEELdkyfknhtspdvd	1310909	vnlttrtqlPPAYTn
1309462	eplvdlpigINITRfqtlla	1311657	ccscgscckfdeddSEPVLkgvkl
1309467	fdeddSEPVLkgvklhyt	1311674	faqvkqiykTPPIKdfggfnfsqi
1309469	fknhtspdvdLGDISginas	1311676	FKEELdkyfk
1309475	gccscgscckfdeddSEPVL	1311813	rlfrkSNLKP
1309492	ILDITpcsfggvsvitpgtn	1314023	ynylyrlfrkSNLKP
1309504	kqiykTPPIKdfggfnfsqi	1314425	ALDPLSEtk
1309506	kvggnynylyrlfrkSNLKP	1316945	fsqilpdPSKPSKrsfie
1309519	lpdPSKPSKrsfiedllfnk	1317060	ftISVTTEi
1309522	lPPAYTnsftrgvyypdkvf	1324400	sFKEELdky
1309555	qcvnlttrtqlPPAYTnsft	1325190	swmesEFRVY
1309558	qfnsaigkiqdsLSSTAsal	1328800	ytmslgAENSVay

[1] Epitopes listed as IEDB ID. [2] Shared peptides given in capital letters.

As a matter of fact, exploration of IEDB reveals that all of the shared sequences − TKTSV excepted − are distributed through 68 immunoreactive SARS-CoV-2 spike gp-

derived epitopes (Table 2), thus documenting the possibility of autoimmune cross-reactions and consequent cardiac pathologies following SARS-CoV-2 infection. Immunologically, data from Tables 1 and 2 offer a mechanism, i.e., cross-reactivity, and a molecular target, i,e., Titin, to explain the still unknown etiology of sudden death occurrence following SARS-CoV-2 infection [6-9].

Moreover, the immunological cross-reactivity potential illustrated in Table 2 may be further bolstered by the mechanism of the immunologic imprinting. In fact, comparative sequence analyses show that 8 out of the 29 minimal immune determinants common to Titin and SARS-CoV-2 spike gp (namely, AGAAA, LEILD, SLLIV, FKEEL, GKLQD, LQDVV, SVTTE, and VEAEV) are also present in microbial agents − *B. pertussis*, *C. tetani*, *C. diphtheria*, and *N. meningitides* − to which, usually, an individual may have been already exposed during his life because of the vaccinal route (Table 3).

Table 3. Occurrence in microbial organisms of the pentapeptides common to Titin, SARS-CoV-2 spike gp, and SARS-CoV-2 spike gp-derived epitopes

Organism	**Shared peptides**
B. pertussis	AGAAA, LEILD, SLLIV
C. tetani	AGAAA, FKEEL, GKLQD, LQDVV, SVTTE
C. diphtheria	AGAAA, VEAEV
N. meningitides	AGAAA, LEILD
Poliovirus 1	−

So, it appears that Table 3 is of cardinal importance since it implies the possibility that, through the immunologic imprinting, a preexisting immune response to a previously encountered pathogen (in the case in object: *B. pertussis*, *C. tetani*, *C. diphtheria*, and *N. meningitides*) might be boosted by a successive SARS-CoV-2 infection. Then, the primary response to SARS-CoV-2 can turn into an anamnestic enhanced secondary response to the previously encountered different pathogen [24-27]. The logical sequelae may be that:

- lower or no immune responses may be evoked against the pathogen lastly encountered, i.e., SARS-CoV-2,
- reactions may occur only against the early sensitizing pathogen (i.e., *B. pertussis*, *C. tetani*, *C. diphtheria*, and *N. meningitides*) that, on the other hand, are no more present in the organism so that
- the anamnestic, high affinity, high avidity immune responses triggered by the pathogen lastly encountered (SARS-CoV-2) may find an outlet by hitting available human targets (in the case in object, Titin).

4 Discussion

For centuries scientists and physicians have studied, discussed, dissected the clinical issue of sudden death without, unfortunately, reaching a satisfactory explanation [28]. Here, the hypothesis has been investigated that infectious agents can induce cross-reactive autoantibodies capable of binding human cardiac proteins thus

functionally affecting them and causing sudden death. Data have been obtained (Tables 1 to 3) by using as a research model SARS-CoV-2 and the cardiac human protein Titin. On the whole, the results support the working hypothesis and invite to analyze the potential cross-reactivity of other infectious agents *vs* additional sudden death-associated human proteins.

Moreover, in cases of sudden death associated with SARS-CoV-2 infection, this study warrants the testing of subjects' sera for autoantibodies against the pentapeptides targets described in Tables 1 to 3. Such an experimental approach might reveal itself as bearing high relevance from a diagnostic point of view. Indeed, as a rule in case of sudden death, post-mortem autopsies do not reveal tissue inflammation, degeneration or alteration that might allow the establishment of a cause [29-31]. Detailed protocols for the examination of the heart and recommendations for the selection of histological blocks and appropriate material for toxicology, microbiology, biochemistry, and molecular investigation have been proposed [32]. However, despite comprehensive macroscopic, microscopic, and toxicological investigation, around 5%-10% of cases may remain unexplained and, in the young population, this percentage may increase up to 30%-50% [33]. "Molecular autopsy", i.e., post-mortem cardiac genetic testing, has also been performed in addition to histology and other analyses, in order to understand the possible genetic causes of sudden death. However, mortality risk associated with truncating founder mutations in Titin was not significantly increased in 20522 person-years, whilst mortality was significantly increased in subjects living after 1965 [34], thus posing the question of whether the mortality increase was related to the introduction of mass immunization in the '60s [35]. However, whatever the answer, it remains the bitter truth of the numbers: already in 2008, the annual incidence of sudden cardiac death in the US was likely to be in the range of 180–250,000 per year, while the global annual incidence of sudden cardiac death was estimated to be in the range of 4–5 million cases per year [36].

Actually, the absence of pathognomonic histopathologic findings and the absence of cardiac pathology find an explanation in the data presented here. That is, since cross-reactive autoantibodies induced by infectious agents can be endowed with high avidity and high affinity, then the cross-reactions and the block of cardiac function and the death will be also extremely rapid, with no time left for degenerative processes to occur so that no cardiac histopathologic alterations can be found. In this context, the data presented here indicate the need of a strict and thorough clinical surveillance on the future effects of the mass vaccination against the current SARS-CoV-2 pandemic.

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