Human Papillomavirus Epitope Mimicry and Autoimmunity: The Molecular Truth of Peptide Sharing

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Keywords

Human papillomavirus L1 epitopes · Peptide sharing · Cross-reactivity · Molecular mimicry · Autoimmunity · Negative selection

Abstract

Objective: To define the cross-reactivity potential and the consequent autoimmunity intrinsic to viral versus human peptide sharing. **Methods:** Using human papillomavirus (HPV) infection/active immunization as a research model, the experimentally validated HPV L1 epitopes catalogued at the Immune Epitope DataBase were analyzed for peptide sharing with the human proteome. **Results:** The final data show that the totality of the immunoreactive HPV L1 epitopes is mostly composed by peptides present in human proteins. **Conclusions:** Immunologically, the high extent of peptide sharing between the HPV L1 epitopes and human proteins invites to revise the concept of the negative selection of self-reactive lymphocytes. Pathologically, the data highlight a cross-reactive potential for a spectrum of autoimmune diseases that includes ovarian failure, systemic lupus erythematosus (SLE), breast cancer and sudden death, among others. Therapeutically, analyzing already validated

immunoreactive epitopes filters out the peptide sharing possibly exempt of self-reactivity, defines the effective potential for pathologic autoimmunity, and allows singling out peptide epitopes for safe immunotherapeutic protocols.

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Introduction

Autoimmunity derives from an immune response directed against proteins/structures normally presented within the body of the host [1]. In general, infections precede the onset of autoimmune diseases [2–9] and, since the 80s, the sharing of sequences/structures between microbial agents and humans has been proposed as the cross-reactive molecular basis for infection-induced autoimmune diseases [10, 11].

During the last two decades, the advent of proteomics allowed comparative biochemical analyses of microbial and human proteomes and highlighted data never reported before, namely the high quantitative dimensions of the peptide commonality among all organisms. Indeed, starting from 2000 [12], numerous studies [13–18] have documented that microbial proteomes have an incredibly high

number of peptide overlaps with the human proteome. The massive and widespread distribution of microbial sequences throughout the human proteome shakes up the immunological concept of a “human self” distinct and separated from “the others,” be they viruses, bacteria, protozoa, plants, and others. Just examining only 30 viral proteomes, biochemical sequence analyses and mathematical calculations highlight a viral-versus-human peptide overlap amounting to 2,907,096 total pentapeptides [13]. Such a peptide commonality nullifies the boundary between the human self and the nonself, that is to say the core issue in immunology and immunotherapy [19, 20], since pentapeptides are minimal sufficient immunological determinants [21–25].

Hence, the awesome dimensions of the microbial versus human peptide overlap raise basic immunotherapeutic concerns. Indeed, how to distinguish, within the massive common sequence platform, the peptide sequences that can be involved in self-reactivity and lead to autoimmune diseases? How to single out peptide sequences that might be useful for immunotherapy against pathogens? As a matter of fact, the unexpectedly high extent of the peptide sharing hampers a peptide-by-peptide immunological characterization and the utilization of the peptide overlap data, thus making the goals of immunotherapy seem unattainable.

To investigate these issues, the present study (i) uses human papillomavirus (HPV) infection/active immunization as a research model since numerous epitopes from HPV variants [26] have been experimentally validated and are available at the Immune Epitope DataBase (IEDB) [27]; (ii) searches the immunoreactive HPV epitopes for peptide matches with the human proteome; (iii) describes examples of human proteins that – when hit by cross-reactions generated by HPV infection/active immunization – may associate with diseases and autoimmune manifestations; and (iv) traces the confinement borders between “safe” and “unsafe” epitopes, namely, identifies HPV sequences that, by being absent in the human proteome, can be safely used for anti-HPV immunotherapy.

Methods

The immunopositive HPV L1 linear epitopes (186 sequences) were retrieved from IEDB (www.iedb.org) [27] and derived from 15 HPV types (1a, 2, 3, 4, 6b, 6, 11, 16, 18, 29, 31, 33, 44, 52, and 58). Each HPV L1 epitope was dissected into pentapeptides, that is minimal immune determinants [21–25], offset each other by one amino acid (aa). The resulting 980 pentapeptides were analyzed for occurrences in the human proteome using Pir Peptide Match program (<https://research.bioinformatics.udel.edu/peptidematch/index.jsp>) [28]. Human proteins that shared peptide sequences with the HPV L1 epitopes were recorded and analyzed for functions and associated pathologies using UniProt database (www.uniprot.org) [29] plus PubMed and OMIM resources.

Results and Discussion

Description of the Peptide Sharing between HPV L1 Epitopes and Human Proteins

Only 95 out of the 980 pentapeptides derived from the 186 HPV L1 epitopes are uniquely present in the viral proteins and absent in the human proteome (Table 1). Aiming at visualizing the complexity and extent of the viral versus human peptide overlap, Table 1 reports shared pentapeptides with initial aa in capital format and HPV-specific pentapeptides with initial aa in small format. For example, the epitope YLKGNNGRET (IEDB ID: 74736) consists of 6 overlapped minimal immune determinants: YLKGNN, LKGNN, KGNNG, GNNGR, NNGRE, and NGRET. All but one of the 6 immune determinants are shared with human proteins and are visualized with the initial aa capital marked. The pentapeptide unique to HPV (NNGRE) is given with the initial aa N in lowercase letters.

The 95 pentapeptides that are uniquely present in the HPV epitopes are listed in Table 2. These 95 HPV L1 pentapeptides are distributed among 71 HPV types (data not shown) and might have immunotherapeutic utilization for active immunizations exempt of cross-reactivity.

At a glance, Table 1 clearly documents the impressively high extent of the peptide sharing occurring between immunoreactive HPV L1 epitopes and the human proteome. In practice, all of the analyzed HPV L1 epitopes are characterized by a viral-human compositional peptide mosaicism that confirms the biochemical evolutionary connection between viruses and eukaryotes [30]. Moreover, it has also to be underscored that Table 1 describes only linear HPV L1 epitopic sequences. Actually, discontinuous nonlinear epitopes add to the cross-reactivity burden and the autoimmune potential illustrated above. For example, a search through IEDB shows that the HPV L1 epitope IEDB ID 114247 corresponds to the discontinuous hexapeptide A¹⁵⁸S¹⁵⁹A¹⁶⁰A¹⁶³N¹⁶⁴A¹⁶⁵, and the HPV L1 epitope IEDB ID 114248 corresponds to the discontinuous peptide L²⁸⁶, A²⁹⁰, A²⁹², N²⁹⁶, D²⁹⁹, T³⁰⁹, N³¹¹, S³¹⁴, N³¹⁶, S³⁷⁵, T³⁷⁶, E³⁷⁸, Y³⁸¹, N³⁸³, T³⁸⁴. And finally, although we do not report on protein isoforms because of space, it has to be mentioned that isoforms, expressed in different tissues and at different times of the

Table 1. Pentapeptide sharing between HPV L1 immunoreactive epitopes and human proteins

IEDB ID ^a	EPITOPE ^{b-d}	IEDB ID ^a	EPITOPE ^{b-d}	IEDB ID ^a	EPITOPE ^{b-d}
24390	HNngiCWGN	111890	VdNReCISMDYKqTQLCLIG	134516	RSGTVG
74736	YLKGnNGRET	111907	VEVGRGQPLGVGISG	134517	RSGTVGESVP
108925	EQmFvRHLFNAGTVG	111909	VGHPYFPIKKPNNNK	134530	SGTVGESVP
109085	GFGAMDF	111910	VGHPYFSIKRANKTVVPKVS	137611	AVPDDLYIK
109331	IHSMNSSIL	111911	VGISGHPLLNKLDDT	137643	FFPTPSGSI
109332	IHSMNSTIL	111915	VHTGFGAMDFTTTLQANKSEV	137654	GTyKNDNFk
110064	VSLGQYRVFRIHLPDP	111924	VSLGQYRVFRIHLPD	137660	KyTfWEVNL
110186	AGVdNRECI	111946	WGNQLFVTVVDTTTS	137665	LqFVFQLCK
110343	NLASSnYFPTPSGSM	111952	YAANAGVdNReCISM	137666	MTLCTEVTk
110375	PSGSMVTSDAQIFNK	111955	YDDVENSggyGgNPGQDNrV	137672	PTTRAPSTK
110428	TDaYVTRTnFYHA	111960	YHAGTSRLAVGHPY	137685	RVRLPDPNK
110629	icWGNQLFV	111962	YHIFQMSLWLPSEA	137686	SIKSPNNNK
110652	LCLIGCKPpigeHWGKGSPC	111965	YkNTnFKeYLRHGEE	137695	STKRKKVKK
110733	VGENVPDDLYIKGSG	112060	cAPAiGeHWAKGTACK	137702	TSESQLFNK
110750	YYHAGTSRL	112061	CAPPLgeHWGKGQTCT	137704	TYiHTMDSN
110761	AENPTNY	112064	cKPpigeHWGKGSPCT	137705	TYRFVTSQA
110856	FSADLDQFPLGRKFL	112200	lcATEASDTNyKaTnFKEYL	137710	VFQLCKITL
110872	GSGSTANLASSNYFP	112316	SIKNPTNAK	137724	yTfWEVNLK
110877	GTVGENVPDDLYIKG	112317	SIYVNTPSGSLVSS	137726	YyYAGSSRL
110910	KAKPKFTLGKRKATP	112339	SVPTDLYIK	146066	VPKVSGLQY
110916	KLDDTENASAYAANA	112352	TGNKYPGYP	147697	FYNPETQRL
110948	MVTSDAQIFnkPYWL	112373	VFHVKLPDPNKFGLP	147941	LQFIFQLCK
110975	QIFnkPYWLQRAQGH	112374	VFKVVLDPdPNKFALP	147981	MTLCAEVKK
111089	ADvmTYIHSMNSTIL	112377	VFRVQLPDPNKFGLP	148116	PYGDSLFFF
111110	ANKSEVPLDICTSIC	112442	ACQKH	148160	QYRVFRIKL
111113	APKEDPLKKyTFWEV	112443	ACqkHTPPAP	148172	RIKLDPDNK
111139	CAAISTSETtYKNTN	112448	AMNFADLQTnKSDVP	148216	SAPRTSTKK
111165	CPPLELINTVIQDGD	112475	DGRENISMDYKQTQL	148234	sFYNPETQR
111175	CTNVAVnPGDCPPLE	112477	dNReCISMDYKQTQL	148267	SSAPRTSTK
111176	CTsICKYPDYIKMVS	112478	dNRQDVSLDPKQTQM	148278	STYKNENFK
111196	DgyVaRtnIYYHAGT	112479	DnRVnVGMDYKQTQL	148487	YYVAGVNVF
111218	DTTrsTNMSLCAAIS	112481	DpYGDRLFFYLrKEQ	149790	KYPdYLqMSADPYGD
111230	eCISMDYKqTQLCLI	112483	DSRQNTAFdAKQTQM	175568	AQIFnkPYWL
111232	EdTYRFVTSQAIAcq	112486	dTYRF	175639	QIFnkPYWL
111272	FGAMDFTTLQANKSE	112487	DtyRYVQsqAITCQK	418489	RLLLVGHPL
111286	FPDTsFYNPDTQRLV	112490	DVRDNVSVdYKQTQL	440042	RlvwACVGV
111287	FPIFLQMAL	112495	ELINTVIQDGdMVDt	558380	AaATDCPP
111304	GAVGENVPDDLYIKG	112496	ELITAPIQDGdMVDt	558384	AKPRLKR
111351	GLQPPPGGTLEDTYR	112497	ELITSVIQDGdMVDt	558389	AVPDDLYI
111416	IFQLCKITLTADVMT	112510	FnkPYWLHKaqGHNN	558392	CPPLELFN
111418	igeHWGKGspCTNVA	112524	GGTLEDtTYRF	558404	DFGTLQAN
111422	IHLDPDNkFGFPDTS	112525	GHPYFSIKRANKTVV	558408	DnFKEY
111424	IKMvSEPYGDSLFFY	112550	IQDGdmVDTGFGAMN	558414	DVPID
111428	IQDGdMVHTGFGAMD	112569	LcSITLSAEVMAYIH	558416	EDGDMV
111502	LNTnFKeYLRHGEEY	112572	LLLLRKEqMFARHF	558422	ESQLFNKP
111510	LPSEATVYLPPVPVS	112573	LLFYLRKEQ	558424	FGTLQAN
111533	LYIKGSGSTANLASS	112599	MFFYLrKEqLFARHF	558426	GHPYLN
111597	nqFPiMLQF	112633	QDTYRYLQsqAITCQ	558433	IEDGDMVD
111604	nVFPiFLQM	112634	QFTNTTIEDGDMVET	558439	KAKPRLKR
111611	pCTNVAVnPGDC	112638	QMIESVIEDGDMMDI	558442	KEKEDPLN
111634	PNNNKILVPKVSGLQ	112654	RVFKVVLDPdPNKFAL	558443	KFDDTETS
111653	PVPVSKVVSTDGYVA	112655	SADLDQFPLGRKFLL	558453	LKRSAPTT
111667	QFPiFLQF	112656	SAEVMAYIHTMNPVS	558460	mVDTGFGC
111673	QLCLIGcKPpigeHW	112663	SIyVHTPSGSLVSSE	558464	NDnFKEYV
111708	RaqGHNngicWGNQL	112676	TniFYHASSRLLAV	558473	PLELF

Table 1 (continued)

IEDB ID ^a	EPITOPE ^{b-d}	IEDB ID ^a	EPITOPE ^{b-d}	IEDB ID ^a	EPITOPE ^{b-d}
111725	RHGEEYDLQFIFQLC	112683	TPSGSLVSSEAQLFN	558480	PNKFGF
111733	RKATPTTSSTSTTAK	112685	TSifyHAGSSRLTV	558485	QLFNK
111785	SLFFYLRRQmFVRH	112730	yKEYMRHVEEFDLQF	558491	rYPAQPGS
111801	SSILEdWNFGVPPPTSLV	112759	cSNNAITPGDC	558523	YLPPVP
111811	STILEdWNFGLQPPPGGTLE	113993	VHTGFGAMDFTTLQ	558524	YPAQPGS
111826	TfWEVNLKEKFSADL	134343	DLYIK	604717	TSDAQIFNKP
111846	TqRlvwACVGVEVGR	134509	RHfFNRSQTV	832586	CISMDYKqTQL- CLIGcKPPIGE

^a Epitopes listed according to IEDB ID number [27]. ^b Epitope sequences given in 1-letter code. ^c Epitope assay details and references are available at www.iedb.org [27]. ^d Pentapeptides shared between HPV L1 proteins and the human proteome are given with the initial aa in capital format, while pentapeptides present only in HPV L1 epitopes are given with the initial aa in lowercase format.

Table 2. Description of 95 HPV L1 epitope-derived pentapeptides that are absent in the human proteome^a

NGICW	MTYIH	KFGFP	CAPAI	DMVDT
GICWG	YTFWE	VSEPY	EHWAK	MVDTG
ICWGN	TYKNT	DMVHT	LCATE	CSITL
NNGRE	NPGDC	NFKEY	CATEA	QMFAR
MFVRH	SICKY	EYLRH	YKATN	QLFAR
VRHLF	GYVAR	NQFPI	ATNFK	DGDMM
DNREC	YVART	QFPIM	NKYPG	YVHTP
NYFPT	ARTNI	PIMLQ	PNKFA	FYHAG
AYVTR	TNIYY	IMLQF	QKHTP	YHAGS
NIFYH	NIYYH	NVFPI	KHTPP	YKEYM
IFYHA	RSTNM	AQGHN	NKSDV	CSNNA
CWGNQ	STNMS	QGHNN	DNRQD	FFNRS
CKPPI	ECISM	DWNFG	NRNVN	QFVFQ
PIGEH	QTQLC	FWEVN	NVGMD	IHTMD
IGEHW	DTYRF	QRLVW	PYGDR	YYAGS
GEHWG	SFYNP	LVWAC	DAKQT	DYLQM
EHWGK	IFLQM	VWACV	TYRYV	QMSAD
KPYWL	SPCTN	WACVG	YRYVQ	AATDC
VMTYI	PCTNV	KNTNF	QAITC	RYPAQ

^a HPV L1 epitopes were dissected into pentapeptides and the resulting 980 pentapeptides were analyzed for occurrences in human proteins using the Pir Peptide Match program [28]. Pentapeptides absent in the human proteome were recorded.

cellular growth, introduce a further factor of complexity in the immunoreactivity scenario and might help explain the different responses of individuals to infections/active immunizations.

The Pathological Implications of the Peptide Sharing between HPV L1 Epitopes and Human Proteins

The shared 885 HPV epitopic pentapeptides are disseminated throughout 4,938 human proteins for a total of

6,964 occurrences, including multiple/repeated occurrences. The unexpected enormous size of the peptide overlap between the HPV epitopes and human proteins can be appreciated by considering that the mathematical probability that one pentapeptide occurs in two proteins is equal to 20^{-5} , that is, 0.000003125. This extremely relevant peptide sharing indicates a cross-reactivity potential capable of triggering an extremely wide and complex spectrum of autoimmune diseases that cannot be analyzed in detail because of obvious reasons of space. Then, confining the discussion to a few human proteins and reporting the shared peptides in parentheses, peptide matching analyses show the pathological scenarios that follow.

Ovarian dysgenesis, anovulation and male infertility, altered gene expression during oogenesis, premature ovarian failure, diminished ovarian reserve, accelerated primordial follicle loss, oocyte DNA damage, as well as susceptibility to breast/ovarian cancer could occur following immune attacks against DNA helicase MCM9 (LLLVG) [31]; histone-lysine N-methyltransferase 2D (GLQPP, VSSEA, HKAQG) and histone-lysine N-methyltransferase 2B (TPPAP, CQKHT, LQPPP) [32, 33]; protein KASH5 (LQPPP) [34]; histone H1oo (AGSSR, KGSGS, SSTST) [35]; protein diaphanous homolog 2 (FRIHL, GVPVP, NKFGL) [36]; breast cancer type 2 susceptibility protein (EEFDL, LKGNN, STILE, TVVDT) [37]; Bcl-2-related ovarian killer protein (VVSTD) [38]; bone morphogenetic protein 15 (KNPTN) [39]; eukaryotic translation initiation factor 4E transporter (HPLLN, PTTSL) [40]; and activin receptor type-2B (YLKGN) [41].

Disorders in spermatogenesis, sperm – egg fusion, or spermatid maturation and male infertility can associate with altered spermatogenesis-associated protein 16

Table 3. Peptide sharing between HPV L1 epitopes and lupus autoantigens^a

Shared peptides	Lupus autoantigens
YHASS	Complement C1q subcomponent subunit B
KATPT, ATPPT, TPTTS	Complement component C1q receptor
FTLGK	Complement C1q tumor necrosis factor-related protein 4
PSGSM	Complement factor B
TPSGS	Complement factor H
RVNVG	Complement C2
PLLNK, RAPST	Complement C3
SRLLA	Complement C4-A
SRLLA	Complement C4-B
GVGIS, SSRLI	CO5
PNKFG	CO8A
PPVPV	CR1L
TPSGS	FHR3
TPSGS	FHR4
STTAK, TVIQD	EEA1
DTENA	ITAM
AQPGS	LA
TPSGS	LARP6
LLLVG	LYAM3, P-selectin
SGSLV	MAGB2
TSSTS	PTN22
SGLQY	TNAP3
VPPPP	TREX1
EVPLD, LDICT	TRNK1

CO5, complement C5; CO8A, complement component C8 alpha chain; CR1L, complement component receptor 1-like protein; FHR3, complement factor H-related protein 3; FHR4, complement factor H-related protein 4; EEA1, early endosome antigen 1; ITAM, integrin alpha-M; LA, lupus La protein; LARP6, La-related protein 6; MAGB2, melanoma-associated antigen B2; PTN22, tyrosine-protein phosphatase nonreceptor type 22; TNAP3, tumor necrosis factor alpha-induced protein 3; TREX1, three-prime repair exonuclease 1; TRNK1, TPR and ankyrin repeat-containing protein 1. ^a HPV L1 epitopes were dissected into pentapeptides and analyzed for occurrences in the human proteome using Pir Peptide Match program [28]. Pentapeptides shared with autoantigens associated with lupus were recorded. The list of lupus autoantigens was obtained using UniProt database (www.uniprot.org) [29] plus PubMed and OMIM resources, and data from ref [56].

(AGSSR) [42]; spermatid-specific linker histone H1-like protein (FTLGK) [43]; spermatid-specific manchette-related protein 1 (ISGHP, GHPYL) [44]; and sperm flagellar protein 2 (KKVKK, RKFLI, SESQL) [45].

Neuropsychiatric diseases including epilepsy, schizophrenia, bipolar disorder, depression, and brain cancer can derive from alterations of 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-1 (KVVLV) [46]; deleted in malignant brain tumors 1 protein (SEVPL, EVPLD) [47]; neuroblastoma breakpoint family member 1 (NLKEK, SSAPR) [48]; neurofibromin (AEVMA, PLLNK, STKRK) [49]; neuronal migration protein doublecortin (LASSN) [50]; neuron navigator 3 (FKEYV, KEKED, LFNKP, SLVSS) [51]; and teneurin-2

(KVVST, LRKEQ, LWLPS, RLLAV) [52]. Actually, the potential immune impact on cell and organ functions might be even more complex because of the frequently multiple cellular functions associated with proteins. For instance, teneurin-2 is implicated in neural development and establishment of proper circuit-wiring within the nervous system, promotes the formation of enlarged growth cone in neuronal cells, induces homophilic cell-cell adhesion, may function as a cellular signal transducer, and increases survival in ovarian cancer patients [53–55].

Lupus manifestations can derive from immune attacks against the lupus autoantigens listed in Table 3 [56], thus justifying recommendations and barriers to vaccination in SLE patients [57, 58].

Fig. 1. Schematic structure of a voltage-gated potassium channel protein: the HPV L1 pentapeptide RVFRI is allocated in the sensor domain S4, which is involved in closing/opening the pore and the K⁺ flux [66].

Altered control of the vascular dynamics, pain, fevers associated with the menstrual cycle, depression, hypotension, and dysregulation of blood pressure may follow immune attacks against D(1B) dopamine receptor (APPLG) [59], D(4) dopamine receptor (TPPAP) [60], 5-hydroxytryptamine receptor 1A (LPSEA) [61], 5-hydroxytryptamine receptor 7 (EVGRG) [62], beta-adrenergic receptor kinase 2 (RSGTV) [63], neurabin-2 or spinophilin (TAPIQ) [64], and sodium/glucose cotransporter 2 (LLVG) [65].

Then, focusing on cardiac autoimmunity and sudden unexplained death, it is of remarkable importance the HPV L1 pentapeptide RVFRI shared with 11 potassium voltage-gated channel proteins (UniProt entries: KCA10, KCNA1, KCNA2, KCNA3, KCNA4, KCNA5, KCNA6, KCNA7, KCND1, KCND2, and KCND3). Voltage-gated potassium channels are proteins characterized by 6 transmembrane segments (S1–S6), 2 of which (S5–S6) form a central pore surrounded by the 4 voltage sensor domains S1–S4. Crucially, the HPV L1 pentapeptide RVFRI is allocated in the sensor domain S4 (Fig. 1), which, upon depolarization, moves outward carrying charged residues across the membrane field, thereby leading to the opening of the pore [66]. Alterations of potassium voltage-gated channel proteins have been related to Brugada syndrome and sudden unexplained death [67].

The set of potassium voltage-gated channel proteins containing the pentapeptide sensor motif RVFRI is additionally flanked by a further group of potassium-channel proteins (Table 4) that might be targeted by cross-reactive anti-HPV immune responses. On the whole, im-

mune attacks against these cardiac targets might lead to channelopathies that play a crucial role in sudden death and in the genesis of atrial fibrillation and cardiac arrhythmias [68], early infantile epileptic encephalopathy [69], and febrile seizure syndromes [70].

Risk of sudden unexplained death is also present when examining the high extent of peptide sharing (i.e., 30 pentapeptides, including multiple occurrences) with Titin (Table 5), a cardiac protein that, when altered, may associate with sudden death [71].

Other crucial cardiac proteins involved in the peptide sharing with HPV L1 proteins are (with shared pentapeptides in parentheses) cardiomyopathy-associated protein 5 (VKLPD) [72]; trans-2,3-enoyl-CoA reductase-like (QMSLW) that associates with arrhythmia [73], and myocyte-specific enhancer factor 2A (PPPGG, PPPPT, and PSGSL) that relates to coronary artery disease [74].

Immune Attacks against One Single HPV Epitope Can Lead to Multiple Diseases

Moreover and of very special relevance in the present context, an immune attack against one single HPV epitope can cross-react with numerous human proteins, each of which is implicated in specific pathologic manifestations (Fig. 2).

In fact, the HPV L1 epitope YDDVENS GG YGGNP-QQDN RV (IEDB ID: 111955) shares the sequence

- YDDVEN with Rho guanine nucleotide exchange factor 10 that is related to slowed nerve conduction velocity and thin myelination of peripheral nerves [75];
- SGGYGG with heterogeneous nuclear ribonucleoproteins A2/B1 that is a target for antinuclear autoantibodies in SLE, rheumatoid arthritis (RA), and autoimmune hepatitis (AIH) [76]; its alteration in Alzheimer's disease impairs cortical splicing and cognitive function [77] and relates to multisystem proteinopathy and amyotrophic lateral sclerosis (ALS) [78];
- SGGYGG with transcription factor Sp7 that is implicated in altered osteogenesis [79], and with keratin, type II cytoskeletal 1b or K1b expressed in skin and hair follicle, so that alteration of K1b may lead to hair loss [80];
- SGGYGG with TATA-binding protein-associated factor 2N, alterations of which are implicated in ALS [81–83];
- SGGYGG with ATP-dependent RNA helicase A, knockdown of which increases the amount of viral-derived circular RNA produced during HBV replication and the viral protein levels [84];
- YGGGNP with SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D

Fig. 2. Immune responses against HPV L1 epitope YDDVENS~~GGYGG~~NPGQDNRV can hit multiple human proteins and lead to multiple diseases.

Table 4. Pentapeptide sharing between HPV L1 epitopes and potassium-channel proteins^a

Shared peptides	Potassium-channel proteins
TVGES	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 1
APRTS	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 2
KGSGS, PPPGG, RAPST	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 3
KAKPK, AKPKF	ATP-sensitive inward rectifier potassium channel 11
NLKEK	ATP-sensitive inward rectifier potassium channel 15
KYPGY, YPGYP	Inward rectifier potassium channel 16
VENSG, VPTDL	Calcium-activated potassium channel subunit alpha-1
PAQPG	Potassium voltage-gated channel subfamily C member 3
LFVTV	Potassium voltage-gated channel subfamily G member 1
GSLVS, SGSTA	Potassium voltage-gated channel subfamily H member 4
TTRST	Potassium voltage-gated channel subfamily H member 8
TYRYL	G protein-activated inward rectifier potassium channel 4
TYRYL	G protein-activated inward rectifier potassium channel 2
TYRYL	G protein-activated inward rectifier potassium channel 3
VAGVN	Potassium channel subfamily K member 5
NAITP, AITPG	Potassium channel subfamily K member 12
VSLDP	Potassium voltage-gated channel subfamily KQT member 1
GKGSP, KGSPC	Potassium voltage-gated channel subfamily KQT member 2
ELITA, PSGSM, TPSGS, RKEQL	Potassium voltage-gated channel subfamily KQT member 3
ELITA	Potassium voltage-gated channel subfamily KQT member 4
ELITA	Potassium voltage-gated channel subfamily KQT member 5
KVVLP, RVNVG, SSRL	Potassium voltage-gated channel subfamily S member 1
GQPLG	Potassium voltage-gated channel subfamily S member 2
TSESQ	Potassium channel subfamily T member 1
TSESQ	Potassium channel subfamily T member 2
VENSG	Potassium channel subfamily U member 1

^a HPV L1 epitopes were dissected into pentapeptides and analyzed for occurrences in the human proteome using the Pir Peptide Match program [28]. Pentapeptides shared with potassium channel proteins were recorded.

- member 1. This protein regulates hepatic fatty acid β-oxidation and its alterations can relate to metabolic syndrome [85];
- GNP~~GGQD~~ with collagen alpha-3(VI) chain precursor, alterations of which are related to dystonia syndrome [86], microstructural white matter abnormalities [87], and muscular dystrophy [88, 89];
- GQDNR with BET1-like protein that associates with uterine fibroid risk [90] and with myelin-associated glycoprotein that can contribute/lead to Charcot-Marie-Tooth 1A disease [91], hypomyelinating leukodystrophy [92], and axial propriospinal myoclonus [93].

Table 5. Distribution of HPV L1 pentapeptides along Titin sequence^a

Pentapeptide	aa position
KTVVP	607–611
TVVPK	608–612
VVPKV	609–613
SLVEE	1,799–1,803
STANL	3,427–34,31
VENSG	5,853–5,857
LELFN	6,877–6,881
STILE	7,908–7,912
KVSGL	8,566–8,570, 22,642–22,646, 26,574–26,578
PPPPT	9,940–9,944
PPPTT	9,941–9,945
VPPPP	10,348–10,352
TEVTK	17,948–17,952
VVDTT	19,432–19,436, 30,277–30,281
DTTRS	20,526–20,530
KRANK	21,150–21,154
RANKT	21,151–21,155
TSRLI	21,441–21,445
SITLS	23,184–23,188, 24,266–24,270
VEETA	25,287–25,291
SLDPK	25,635–25,639
SEVPL	30,559–30,563
KSDVP	31,847–31,851
NKFGL	32,125–32,129
VGENV	32,630–32,634
KKYTF	32,664–32,668

^a HPV L1 epitopes were dissected into pentapeptides and analyzed for occurrences in the human proteome using the Pir Peptide Match program [28]. Pentapeptides shared with Titin were recorded and the aa positions along the Titin primary sequence (<https://www.uniprot.org/uniprot/Q8WZ42>) were annotated.

The Immunological Implications of the Peptide Sharing between HPV L1 Epitopes and Human Proteins

Immunologically, the present data dismantle the main argument against autoimmunity. Indeed, it is assumed [19, 20] that lymphocytes with specificity for peptides that are expressed in the human host do not proliferate and are deleted from the immunological repertoire during fetal or early life in order to avoid self-reactivity. Based on this assumption, cross-reactivity and the consequent autoimmune diseases have been and are considered as rare phenomena caused by an incomplete negative selection of self-reactive lymphocytes that produces the so-called “immunological holes” [19, 20, 94]. Accordingly, considering viruses as triggers of autoimmunity has been defined to be more a fantasy rather than a fact [95]. In conflict, we document here that the totality of the experi-

mentally validated immunoreactive HPV L1 epitopes is mostly composed by peptides present in human proteins. This means that immune attacks against such HPV L1 epitopes concretize a real risk of cross-reactions with the human proteins that share the epitopic peptide sequences and are a prelude to autoimmune pathologies. Furthermore, it appears that the self-reactive lymphocytes that produced the immune responses against the 186 HPV epitopes analyzed here have never been eliminated. In reality, it seems that the process of negative selection of self-reactive lymphocytes does not exist at all.

Rather, the widespread peptide commonality configures a non-reactivity status in almost all lymphocytes from fetal development to final differentiation, so that anti-pathogen (and cross-reactive) immune responses can occur only in the presence of stimuli such as adjuvants [96], lipopolysaccharides [97], altered glycosylation patterns [98], posttranslational modifications as citrullination [99], and chemical modification as oxidation [100] and glycation [101]. In this context, peptide commonality might also explain the silent “immunotolerated” chronic pathogen infections [102] and, as well, the vaccinology failure to induce powerful, specific, and safe immune responses against infectious agents [103]. In essence, peptide sharing with microbial proteins and, in general, with all proteomes is a condition intrinsic to human proteins and reifies immunotolerance, whereas immunogenicity resides in and identifies with peptide sequences that are extraneous to the human host (Table 2) [104].

Conclusions

This study shows a massive viral versus human peptide sharing that involves immunoreactive HPV epitopes and a highest number of human proteins that may be associated – when inhibited, deleted, mutated, modified, or improperly functioning – with pathologies and autoimmune disorders. Specifically, the present data indicate that, via cross-reactivity, the immune responses that follow HPV infections/active immunizations might lead to premature ovarian failure, oocyte DNA damage, lupus manifestations, susceptibility to breast/ovarian cancer, neuropsychiatric diseases, hypotension and dysregulation of blood pressure, cardiac disorders, and, even, sudden death. Clinically, such a vast cross-reactivity potential explains the multiple autoimmune disease syndromes that can follow infections/active immunization [105]. From the immunological point of view, the data pose fundamental questions on the negative selection issue and warrant further re-

search. Moreover and of note, the here described analysis of experimentally validated immunoreactive epitopes not only highlights the effective cross-reactive potential capable of leading to pathologic autoimmune sequelae but also defines a scientific procedure for identifying peptide sequences to be used for safe immunotherapeutic protocols exempt of adverse events [106–108].

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

D.K.: declares no conflicts. Y.S.: is a medical consultant in vaccine compensation court, USA.

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