Peptide Sharing between Parvovirus B19 and DNA Methylating/ Histone Modifying Enzymes. A Potential Link to Childhood Acute Lymphoblastic Leukemia

Anna Polito[1], Riccardo Polimeno[2] and Darja Kanduc[3,*]

[1]*Complex Structure of Neuropsychiatry Childhood-Adolescence, Ospedali Riuniti of Foggia, Foggia, Italy*

[2]*Section of Sciences and Technologies of Laboratory Medicine, Department of Interdisciplinary Medicine, University of Bari, Bari, Italy*

[3]*Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Bari, Italy*

Abstract: The present study investigates the hypothesis that the immune responses that follow active infections may crossreact with (and damage) molecules related to DNA methylation and histone modification, in this way determining the aberrant gene expression so often reported in acute lymphoblastic leukemia (ALL). We used Parvovirus B19 - a pathogen that has been repeatedly studied in ALL – as a model and analysed the viral polyprotein for peptide sharing with human proteins involved in gene expression. Data are reported that document an ample peptide sharing between Parvovirus B19 and human DNA/histone methylation and modification enzymes. Remarkably, the shared peptide platform is endowed with a high immunologic potential**.** This study calls attention on immune cross reactivity as a molecular mechanism that may connect infections to cancer and warns against active immunizations based on entire viral antigens.

Keywords: Peptide sharing, immune crossreactivity, DNA methyl transferases, histone methyl (acetyl) ltransferases, aberrant gene expression.

1. INTRODUCTION

In western countries, cancer is the 2nd cause of death among children, with acute lymphoblastic leukemia (ALL) as the most common childhood leukemia subtype [1, 2].

In studying T- and B-ALL syndromes, the scientific-clinical research has focused on chromosomal translocations [3-9], gene mutations [10-13], and, more recently, on aberrant gene expression, which mainly depends on the methylation status of DNA and histone protein modifications [14-27]. Moreover, infections have been explored and proposed as contributing causal factors [28-35]. In fact, increasing epidemiological evidence [36-45] suggests that infections are involved in the etiology of ALL and calls for research on the molecules and mechanisms that might link infectious agents to all.

In this scientific-clinical frame, we investigated the hypothesis that crossreactivity may represent the mechanism linking Parvovirus B19 infection and cancer. Indeed, over the last decades we found that a vast and massive peptide commonality joins viruses and human proteins [46-48], thus attesting a common evolutionary history [46]. Such a peptide commonality may provide a platform for crossreactions during immune responses following infections and, actually, also has a pathologic significance since often the shared sequences are present in epitopes that have been experimentally validated as immunopositive in humans [49, 50]. Therefore, it appears logical to hypothesise that peptide sharing can trigger harmful autoimmune cross-reactions in the human host.

Pursuing this rationale, we analyse here Parvovirus B19, which has been related to ALL [51-60], and human proteins involved in DNA methylation/histone modifications, searching for shared peptides that might lead to harmful cross-reactions thus possibly causing the disrupted gene expression so often reported in ALL [14-27].

2. METHODS

The amino acid (aa) sequence of Parvovirus B19 (B19; Tax Id: 10798, GenBank: AF162273.1; 2006 aa) was dissected into 1994 pentamers sequentially overlapping each other by four residues, i.e., MSKES, SKESG, KESGK, ESGKW, and so forth. Hemagglutinin (Q67010, 565 aa long) from influenza A H1N1 virus (Tax ID: 382845) was likewise dissected into 5-mers overlapping by four residues (i.e., MKARL, KARLL, ARLLV, RLLVL, and so forth) and used as a viral protein control.

*Address correspondence to this author at the Dept Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Bari, Italy; Tel/Fax: +390805231522; Email: dkanduc@gmail.com

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In parallel, a set of 108 reviewed human protein entries was retrieved from UniProt KB Database (http://www.uniprot.org/) [61] using as keywords 'DNA methyltransferase' and 'histone methyltransferase' (see Supplemental Table **1**).

Table 1: Pentapeptide Sharing between Parvovirus B19 and Human Proteins Related to DNA/Histone Methylation and Modification

Shared Peptide(s)[1]	Human protein[2]
GGGGS; SSSSS; SSSGG	AEBP2. Zinc finger protein AEBP2
PYTHW	ANM1. Protein arginine N-methyltransferase 1
GPLVN	ANM5. Protein arginine N-methyltransferase 5
KESGK; SSDAG; ELSES	ASH1L. Histone-lysine N-methyltransferase ASH1L
GAAAP	ASH2L. Set1/Ash2 histone methyltransferase complex subunit ASH2
EPLTH	CARM1. Histone-arginine methyltransferase CARM1
AKKPR; KKPRI	CDC73. Parafibromin
SSSSS	DMAP1. DNA methyltransferase 1-associated protein 1
AKKPR	DNM3A. DNA (cytosine-5)-methyltransferase 3A
VFTED; EELSE	DNMT1. DNA (cytosine-5)-methyltransferase 1
PAASS; FDLVA; RSSTP	DOT1L. Histone-lysine N-methyltransferase, H3 lysine-79 specific
VKLLL	EHMT1. Histone-lysine N-methyltransferase EHMT1
ENLEG; TGAGK; NDTSS; NSGGG; SGGGL	EHMT2. Histone-lysine N-methyltransferase EHMT2
TKEGD; KEGDS	EZH1. Histone-lysine N-methyltransferase EZH1
PVSQP; SGTGA; QAQVV	HCFC1. Host cell factor 1
SPAAS; EQLKQ; SSVSS	HDAC9. Histone deacetylase 9
YKVFS	HM20A. High mobility group protein 20A
AEDKE	HS90A. Heat shock protein HSP 90-alpha
AEDKE; GKSLV	HS90B. Heat shock protein HSP 90-beta
AVPGV	JARD2. Protein Jumonji
GGGGS; AKKPR	KAT2B. Histone acetyltransferase KAT2B
SSSSS; GGGGS; KVFSP; KATGR; SLLDL; LMKKI; SSGGE; SSVSS	KMT2A. Histone-lysine N-methyltransferase 2A
GAGGG; AGGGG	KMT2B. Histone-lysine N-methyltransferase 2B
SEESA; PGPLV; PNLDD; PQSGP; ISLDN; ALSSS; LSSSS; LLSSS	KMT2C. Histone-lysine N-methyltransferase 2C
FSPLE; SHGQL; GGPLA	KMT2D. Histone-lysine N-methyltransferase 2D
TSQNT; SGGES	KMT2E. Histone-lysine N-methyltransferase 2E
DSFKT; ELLVG; HINNS	KMT5B. Histone-lysine N-methyltransferase KMT5B
ELLVG	KMT5C. Histone-lysine N-methyltransferase KMT5C
ALSSS; AGPPQ; VSSNV	NCOA6. Nuclear receptor coactivator 6
GLLTE	NR1H4. Bile acid receptor
PKFRS; GTSQN; TSNGD; PGTSS	NSD1. Histone-lysine N-methyltransferase, H3 lys-36 and H4 lys-20 specific
SVASK	NSD3. Histone-lysine N-methyltransferase NSD3
SKESG; KESGK; KIVKL; IVKLL	PAXB1. PAX3- and PAX7-binding protein 1
SSSSS; SVPVY	PHF8. Histone lysine demethylase PHF8
MWSEG; GSSVS; SSVSS	PPHLN. Periphilin-1
YSQLA	PRD12. PR domain zinc finger protein 12
GGGGS; TFKLG	PRD13. PR domain zinc finger protein 13
LSSSS; SSSSS; SSEEL	PRDM2. PR domain zinc finger protein 2
GAGGG;AGGGG; GGGGS; DEELL	PRDM8. PR domain zinc finger protein 8
GYSTP; MSKES	RARA. Retinoic acid receptor alpha
SVQLP	RBBP4. Histone-binding protein RBBP4
DVAGK; ISSSG	REST. RE1-silencing transcription factor
SSSSS; LSSSS	SET1A. Histone-lysine N-methyltransferase SETD1A
QPPPQ; LSSSS; SSSSS; PVAHF	SET1B. Histone-lysine N-methyltransferase SETD1B
DEELL	SETB2. Histone-lysine N-methyltransferase SETDB2
YDPEH; LELIQ; SSEEL	SETD2. Histone-lysine N-methyltransferase SETD2
SGTGA	SETD3. Histone-lysine N-methyltransferase setd3
ADEEL	SETD7. Histone-lysine N-methyltransferase SETD7
LSSSS	SIR1. NAD-dependent protein deacetylase sirtuin-1
VLDCA	SMYD3. Histone-lysine N-methyltransferase SMYD3
GGGGS; SSSSS	SUZ12. Polycomb protein SUZ12
TAKSR; AKSRV; ALSSS; ELSES	TASOR. Protein TASOR
DLELI; ENPSS; KALKE	ZHX1. Zinc fingers and homeoboxes protein 1
GGPLA	ZN304. Zinc finger protein 304
DTLGG	ZN335. Zinc finger protein 335

Then each viral pentamer was analyzed for occurrence(s) within the set of the 108 human proteins, and proteins hosting viral matches were recorded. The peptide sharing was investigated for immunologic potential using the Immune Epitope Database (IEDB; www.iedb.org) resource [62]. Only epitopes experimentally validated as immunopositive in the human host were considered.

3. RESULTS

According to literature [63-68], a pentapeptide is a sufficient minimal determinant for epitope-paratope interaction and can act as an immune unit, thus playing a crucial role in cellular immunoreactivity and antigen-antibody recognition. Hence, pentapeptides were used as probes to define the peptide commonality between B19 and human proteins related to DNA and histone modification utilizing Influenza. A hemagglutinin as a control (see Methods).

3.1. Quantitative and Qualitative Analyses of the Peptide Sharing between Parvovirus B19 and Human Proteins Related to DNA/Histone Methylation and Modification

Figure **1**, panel B, shows that 125 parvovirus pentapeptides are distributed in 55 out of the 108 human proteins that directly or indirectly play a role in DNA/histone methyltransferase and modification (see Table **S1** for details).

It can be seen that the extent of the peptide overlap is extremely high when considering that the theoretical probability of a sequence of 5aa occurring at random in two proteins may approximately be calculated as 1 out of 20^5 (or 1 out of 3,200,000), assuming that all aa occur with the same frequency and neglecting protein length. Moreover, the distribution of B19 pentapeptide throughout the 55 human proteins is independent of the protein length. For instance, the histone-lysine N-methyltransferase EHMT2 (1210 aa) shares 5 pentapeptides with B19, whereas the histone-lysine N-methyltransferase KMT2D (5537 aa) has 3 matches. As regards hemagglutinin, the peptide sharing with human proteins amounts to 40 pentapeptides that are distributed among 30 human proteins (Figure **1**, panel A, and Table **S1**). Such peptide sharing is of minor relevance when compared to the Parvovirus B19 peptide sharing; still it appears to be significant and might help evaluate the relationship between exposure to influenza infections and risk of leukemia in young children [69].

Qualitatively, the peptide sharing between Parvovirus B19 and the proteins related to DNA/histone methylation and modification is described in Table **1**.

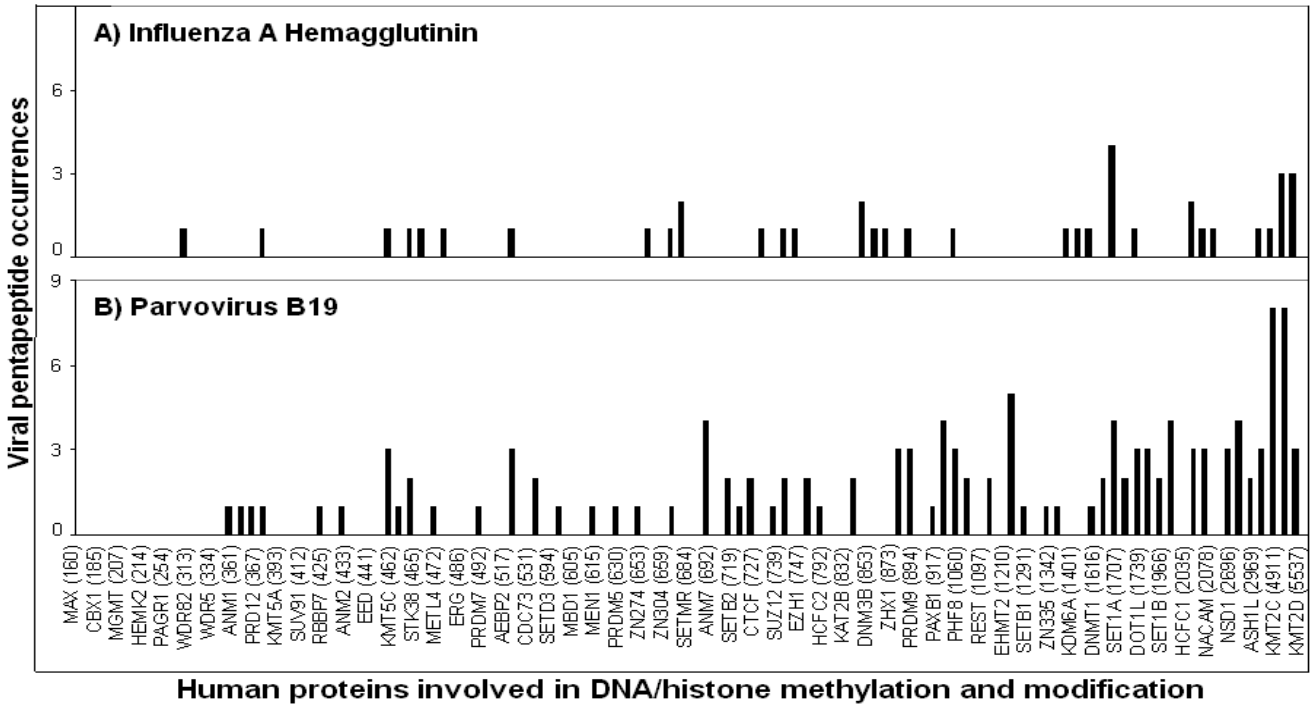

Figure 1: Pentapeptide overlap between Parvovirus B19 polyprotein and 108 human proteins related to DNA/histone modification. Proteins are: indicated by UniProt KB/Swiss-Prot entry names, listed left to right according to increasing length, and described in detail in Table **S1**. Details and references for involvement in gene expression at http://www.uniprot.org/.

Table 2: Immunopositive Epitopes Containing Sequences Shared between B19 and Human Proteins Involved in DNA/Histone Modification

IEDB ID[1]	Epitope[2]	IEDB ID[1]	Epitope[2]
798	adyeelreqlSSVSSferfe	51554	qlSSVSSferfeifpkessw
1432	aGAGGGaggagag	52333	qSHGQLsdhphALSSSSSha
1433	aGAGGGaggaGAGGGaggag	52804	qvvkdyftlkGAAAPVAHFq
1434	aGAGGGaggaGAGGGaggaga	54566	rlgvpDTLGGdPKFRSlthe
1518	aggaGAGGGagga	56758	saENLEGnggpg
1573	agkaltglstGTSQNTrislrp	61638	stGTSQNTrislrpgPVSQP
2788	alnlfFSPLEfqhlienygs	61844	stpwryldfnalnlfFSPLE
4420	asgkeakvctispimGYSTP	62900	TAKSRVhpl
4454	ashlpyieqgmqlaEQLKQk	63887	tglstGTSQNTrislrpgpv
5346	avgimtvtmTFKLGprkatg	63973	tgtDLELIQilkdhynisld
7517	dagesSGTGAevvpfn	64547	TKEGDSsnTGAGKaltglst
7818	ddldegiekSSEELSEek	64815	tlggdPKFRSlthedhaiqp
12111	efyekvtgtDLELIQilkdh	64978	tlnkdqLLSSSkytiqrstg
12901	ekvtgtDLELIQilkdhyni	67298	twtatlcqnlGAAAPe
13350	emavfGAAAPylrks	68237	vekelkdtlENPSSsldegk
16668	flipYDPEHhYKVFSPAASS	68567	vfSPAASSchnasgkeakvc
17073	fmPGPLVNsvsTKEGDSsntgag	69435	vlDSFKTwl
17839	fsrqflipYDPEHhYKVFSP	70182	vnsfdSSSSSdslyesi
18587	GAGGGagaggaGAGGGGrgr	70275	vpDTLGGdPKFRSlthedha
18588	GAGGGaggaGAGGGaggaga	70677	vqyavgimtvtmTFKLGprk
18595	gagkaltglstGTSQNTris	71350	vtgtDLELIQilk
19119	gdssnTGAGKaltglstgts	73164	wtvADEELLkniknetgfqa
19674	ggaggaggaGAGGGag	73804	yfdvNSGGGl
19723	ggeSSEELSESsffnlit	74059	ygnAEDKEyqqgvgrfpnekeq
19737	gggagaggaGAGGGGr	74084	ygsrlgvpDTLGGdPKFRSl
19738	gggaggaGAGGGaggag	74527	YKVFSPAASSchnasgkeak
19814	GGGGSnpvksMWSEGatfsa	74601	yldfnalnlfFSPLEfqhli
20372	gimtvtmTFKLGprKATGRw	75203	ynplygsrlgvpDTLGGdpk
21912	gqlsdhphALSSSSShaepr	78127	edassststsssfpssfpSSSSS
22545	gstmmqtktfSSSSSSkkmg	95214	adyeelreqlSSVSSfer
23542	haKALKErmv	95364	eqlSSVSSferfeifpke
23873	hftsaENLEGng	96063	AEDKEddaed
23985	hhfqSHGQLsdhphALSSSS	96212	eeAEDKEdda
27498	imGYSTPwryldfnalnlff	96215	eeeeAEDKEd
27573	imtvtmTFKLGprKATGRwn	97642	sglvgdtprkndSSSSSh
29879	kaltglstGTSQNTrislrp	97876	ALSSSlgnv
31797	kleaeaKALKEq	103645	tgGGGGSgfsnsgsg
31798	kleaeaKALKEqlakqaeela	104637	srvSSVSSqfsdaaqaspsshsst
34757	laEQLKQkalgllqtatkqa	104638	SSVSSqfsdaaqasp
34959	laSEESAfyvlehssfqllg	105824	ggaGAGGGaggaGAGGGaggag
35244	ldnplENPSSlFDLVArikn	106872	SSSSSgsgsg
36818	lkdhynISLDNplENPSSlf	106878	stSSSSSgsg
36893	lkilPQSGPiggiksmgitt	106879	ststSSSSSg
37300	llfsllgLSSSSSis	106931	tSSSSSgsgs
38659	lPQSGPiggiksmgittlvq	113124	qfqlvesgggl
39164	lrpgPVSQPyhhwdtdk	113351	eaGAGGGa

Table 2 Continue...

IEDB ID[1]	Epitope[2]	IEDB ID[1]	Epitope[2]
39483	lSLLDLylgrgynvs	113375	eqlSSVSSferfe
40495	lvnsvsTKEGDSsnTGAGKa	114797	enPVAHFfknivtpr
40948	lyshhfqSHGQLsdhphals	115386	naneeeysigssSEESAeva
42581	MSKESGKwwesddkfakavy	115479	SEESAevateevkitvddkh
42603	mslSLLDLylgrgyn	118889	lplgqltNSGGGfggvsnal
42883	mtsvnsaeastGAGGGGSnsvk	119738	aGAGGGaggaGAGGGaggagc
43702	nenlddldegiekSSEELSEeki	119876	fseTGAGKhvpra
44350	nlSLDNplENPSSlFDLVAr	119877	fseTGAGKhvpravf
44649	nlamaiakSVPVYgm	119893	ggGGGGSGGGGSgggGGGGSs
45398	nplygsrlgvpDTLGGdpkf	120221	ssssAGGGGgGAGGGGGGGGSgg
45480	npsslFDLVAriknnlknsp	123731	SEESAevateev
46753	PAASSchnasgkeakvctis	124186	gftpGGGGS
47211	pDTLGGdPKFRSlthedhai	127989	ggaGAGGGagagg
48307	plENPSSlFDLVAriknnlk	127990	ggaGAGGGaGAGGGag
48879	pppqiflkilPQSGPiggik	131001	pierVKLLLq
48896	ppqpeyDLELltscs	131892	gNDTSSvsgwgdpkp
49011	pqiflkilPQSGPiggiksm	133060	eaKALKEql
50108	pyieqgmqlaEQLKQkalgl	134313	AEDKEnykkf
50296	qakleaeaKALKEqlakqae	136122	aGAGGGaggaGAGGGaggagg
50820	qfvefyekvtgtDLELIQil	136863	PGTSSradpgperpprqtp
50917	qgmqlaEQLKQkalgllqta	142396	denPVAHFfknivtprtpp
51063	qiflkilPQSGPiggiksmg	142502	aAGGGGStdnlsy
51553	qlSSVSSferfeifp	142672	GGGGStdnlsytql
143034	rkrrsastaaAGGGG	434256	SLLDLhtkv
143060	sastaaAGGGGStdnl	436273	afggsggrgSSSGGgy
144688	idyeelreqlSSVSSferfe	437611	gGGGGSfGAGGGfgsrsl
146369	kefdGKSLVsv	437618	ggsggsyGGGGSgggygggsgsr
147092	lkesgvkpgqfaaivGAGGGl	437620	ggSSSGGgygsGGGGSssvk
151026	lreqlSSVSSferfe	437633	gilnpsqpgqSSSSSq
152919	qlSSVSSferfeifpktssw	437668	GLLTEirav
153382	faenkkdhfspGGGGS	437997	hpiSSEELlslky
153420	mdwinpfpnfNSGGGs	438540	kENPSSqyw
155542	qrvadicrRSSTPlildtsg	439063	lmikENPSSqy
155863	stplildtsggglqhissgv	440752	srevftSSSSSSS
157436	itqPGPLVpl	441051	TGAGKhvpravf
158384	tqPGPLVpl	441180	tSSSSSSSrqtrpilk
162271	fvADEELvhl	441228	vADEELvhl
162470	ieKEGDSia	441565	vtfSEESAvpk
162618	kefdGKSLV	446299	lpeAKKPRl
169405	nlalsikynKEGDSm	446419	lpSSSSSrgsl
176883	AEDKEnykkfy	446950	nprdpSSSSSm
181124	lnedhwasreNSGGG	448175	semEQLKQkl
188658	dielVKLLL	448938	SSSGGgggggrf
195923	rwlwgflsrnKKPRl	449202	thlSSSGqnl
196220	wlwgflsrnKKPRlc	453413	eeselVKLL
201207	ALSSSVSSsk	454071	GGGGSrselvi
203477	avlsSSVSSk	454978	iSLLDLplsl

Table 2 Continue...

IEDB ID[1]	Epitope[2]	IEDB ID[1]	Epitope[2]
206273	dvpvegSSSSSSSStstvapank	456587	lpSSSGGarl
218984	SEESAvpkrsw	457357	peAKKPRl
219024	sehlSSSGkky	458716	SEESAvpersw
219058	selAEDKEny	459065	SLLDLeqkl
222761	hpiSSEELl	459066	SLLDLgeval
222955	kefdGKSLVsvt	459067	SLLDLplsl
223979	semagppqm	459377	spgnLSSSSl
227016	aGAGGGagaggagga	459393	sphlPGTSSa
227204	gggaggaGAGGGagg	460143	tVKLLLlga
229806	SSSSSeeivpnsaeq	465960	gpalPGPLV
230150	ldawvDTLGGdykakfetfk	466926	ivdsgggllk
239392	allAGGGGppak	467986	lPAASSlktpgtsl
239959	laGAGGGGaavtv	470616	SLLDLddfnpvst
240204	qvvesggglvqp	470617	SLLDLllptgl
240212	reapgpAGGGGGGSrw	470927	spiSSSSSql
240571	tgggGGGGSggtrm	471289	ssedfnmgSSSSSqy
241608	apafanrvrkpnpamPAASS	473606	yldlVKLLL
241989	pnpamPAASShaiasdfass	474611	aasSPAASv
243940	fppSSEELqankatlvcl	475033	aehSLLDLp
247579	adGAGGGdgsagedl	475205	aENLEGkvisl
402582	vadGAGGGdgsaged	475767	ALSSSqaev
418157	gtvellGGPLAhpfqpl	475768	ALSSSrptk
418485	rggcgvvGGGGScssv	475910	apSSSSSSl
422404	hSSEELrnlgw	476137	asqKALKEk
425055	gsnfGGGGSy	476163	aSSSSSqlk
425128	gviSSEELplyy	476825	DEELLgdghsy
425938	kvVKLLLry	478409	gEELSEanvrl
426446	MWSEGryey	478519	getgGGGGSal
427412	snfGGGGSy	478579	ghfaGSSVSf
428158	vLLSSSfvy	478938	gtlPVSQPk
430103	fselAEDKEny	479424	hpiSSEELlsl
430104	fselAEDKEny	481133	kSKESGstl
430230	ftelAEDKEny	483493	qpSSSSSkrsl
430231	ftelAEDKEny	484016	rgilkrnSSSSStds
431520	nlEELSEmry	484222	rlGGGGSpr
432005	qtdprAGGGGggdy	486431	spSSSSSgsl
432006	qtdprAGGGGggdy	486737	stiaSSSSSqlk
432324	SSEELlslky	488029	veaSEESAl
432325	SSEELplyy	488030	veaSEESAlnhl
432361	staptEPLTHwy	488260	vlrGGGGSpr
432496	tDLELIky	490898	eriDLELIsf
432734	ttDLELIky	491652	grldkELSESy
432857	viDEELLgdghsy	494349	rriPVSQPgm
433024	vseSHGQLsy	494425	rrliNSGGGvrf
494620	rrvlvTGAGKgigr	551939	gGGGGSfGAGGGfgsr
495980	vyLSSSSGGsssf	551992	ggSSSSSSygsgrrf
504691	ayggGGGGSsy	552034	gilnpsqpgqSSSSSqtf

Table 2 continue...

IEDB ID[1]	Epitope[2]	IEDB ID[1]	Epitope[2]
504856	dISSSGvnl	552449	hGAGGGafpasqtpskpa
504946	eagppqpmv	552594	htkLSSSSSitltlp
505262	fkSPAASSf	552751	ikdgvSSEELgl
505711	grAGGGGpg	554828	PAASSsrpvaps
506058	ikENPSSqy	555100	qggyggSSSSSSygsgrrf
506924	lpTGAGKsl	556448	SSSGGgggggrfsss
508461	sshsnSSSSSl	556462	SSSSSSygsgrrf
508778	tpSSSSSlqks	557300	vgeQAQVVi
509875	lpLSSSSSv	561942	aaAGGGGgggry
510982	AGGGGggaaaagray	561943	aaAGGGGgggryy
513341	dPAASSsaedslsda	563540	fseTGAGKhv
514083	eaaagaGAAAPasqhp	563941	hvvSSVSSf
514084	eaaagaGAAAPasqhpa	564141	KIVKLkhfek
515031	epaSPAASisrlsgeq	564382	kviSSSSSfak
516567	GGGGSggiaeagsghm	564554	maaAGGGGgggry
516615	ggsvSSVSSsrlq	564605	mviDEELLgdghsy
517055	gpfrsnsISLDNI	565687	SVASKalqk
517056	gpfrsnsISLDNIk	567600	fvADEELvhll
517057	gpfrsnsISLDNIkps	567638	GGGGSyndfgny
517146	gppayhetlagGAAAP	568564	tlhpiSSEELl
517550	gsryiAGGGGtgsig	568758	vvkdlSSEEL
517845	hetlagGAAAPypa	570614	apTGAGKtnval
517846	hetlagGAAAPypas	572208	eprgvsNDTSSl
521363	llesggglvqpggslr	575315	mpTGAGKsl
522260	lvesggglvqpggsl	576924	rpsgSSSSSgvl
522261	lvesggglvqpggslr	577031	rqLSSSSSy
523708	ppayhetlagGAAAPypa	577381	rvlvTGAGK
526872	sryiAGGGGtgsig	577774	sllvGPLVNk
527848	tpgePAASSsrpvaps	577775	sLMKKItl
528541	vdPAASSsaedslsdaa	577809	SPAASraattt
528542	vdpaISSSGpagsyr	578417	SVQLPkpvhk
528648	vesggglvqpggslr	582810	GAAAPyllkk
529013	vlIELLVGi	584127	iiwlpTGAGK
530196	yhetlagGAAAPypa	584381	kAGGGGlvagk
530197	yhetlagGAAAPypas	584505	kefdGKSLVs
530198	yhetlagGAAAPypasq	585679	laaGSSVSltl
530199	yhetlagGAAAPypasqpp	585769	leaGLLTEkqk
532132	hpiSSEELlslk	585937	lgsqQAQVVl
532349	kavpkediysggGGGGSr	586810	pdlGSSVSarr
533143	nPAASSnhw	586905	pgprtSSSSSf
534612	gvigspaanapdagppqrwf	587037	plhSSEELlsl
534618	pdagppqrwfvvwlgtannp	587260	prGAAAPlhi
535830	fselAEDKEnykkfy	587416	pryPAASSl
538430	yaqpsereaGAGGGa	587746	qpldtvSSEEL
540949	agglggGAGGGGdhad	588840	rprpmSSEEL
541118	ALSSSSirv	589710	sAKSRVaff
541643	eAGGGGafivlp	590153	SSSSSgkvihr
544299	nqggyggSSSSSSygsgrrf	590154	SSVSSvvvv

Table 2 continue...

IEDB ID[1]	Epitope[2]	IEDB ID[1]	Epitope[2]
544315	nSSSSStdsetlry	594854	deSSSSSlhatrty
544490	pSSSSSpgagvssyisqpgglhpl	595343	fsfkSPAASSf
544563	qhgggggggggagaAGGGG	595662	gilnpsqpgqSSSSSqt
545294	sLLSSSvlt	595899	gtlPVSQPksw
545356	snanPQSGPppr	595992	gyggSSSSSSygsgrrf
545421	sqgcssggGGGGSsaggsgns	596970	iSSEELlslky
545479	SSSSStdsetlry	597614	kglpgLLSSSw
545514	SVASKhaiyav	598110	ksySSGGEdgy
547429	fppSSEELqankatlvclm	598445	kVKLLLkelw
547599	lfppSSEELqankatlvclisdf	599352	ltAEDKEllw
547801	SSSSSSSSSSSSSSdg	600226	PGTSSvahgfli
549474	gfgndgsnfGGGGSy	600406	qgggGGGGSvpgier
549631	ngfgndgsnfGGGGSy	602116	SSSSSlhatrty
550080	aiteqDLELlkeretai	602117	SSSSSygsgrrf
551558	fakprnqggyggSSSSSSy	602217	stspesPYTHW
551606	ffseTGAGKhvpr	602523	tgfstspesPYTHW
551607	ffseTGAGKhvpra	603232	VLDCAfydpthaw
551608	ffseTGAGKhvprav	608771	hpiSSEELl
551832	GAGGGfgsr	609177	kefdGKSLVsv

[1] Epitopes listed according to IEDB ID number. Only epitopes that had been experimentally validated as immunopositive in the human host are considered. Details on immunoassays and immune context, and references are available at www.immuneepitope.org/.

[2] Peptide sequences shared between B19 polyprotein and human proteins in capital letters.

3.2. Immunologic Potential of the Peptide Sharing between Parvovirus B19 and Human Proteins Related to DNA/Histone Modification

The high extent of the viral *vs* human peptide overlap reported in Figure **1** and Table **1** is of pathologic importance when analyzed in the immunologic context. Indeed, exploration of IEDB highlights that the peptide fragments shared between B19 and human proteins (Table **1**) intensively recur in 510 immunopositive epitopes that have been experimentally validated in humans and that, in part, are reported in Table **2**. Exceptions are DVAGK, HINNS, SGGES, SSDAG, TSNGD, VFTED, and VSSNV, which were not found in the IEDB epitope catalogue.

CONCLUSIONS

The data shown in Tables **1** and **2** outline a wide viral-vs-human peptide commonality and suggest that cross reactivity with human proteins involved in DNA methylation and histone modification — eg, human proteins that play important functional roles in crucial processes such as gene expression, acetylation, alternative splicing, regulation of apoptosis, and regulation of transcription — might represent a mechanism through which B19 infection triggers ALL disease in the human host. In fact, in light of the scientific literature on the role of aberrant DNA methylation and histone modifications in leukemia ethiopathogenesis [14-27], such immune cross-reactions and the consequent disrupted gene expression could contain the key for understanding the harmful relationship between Parvovirus B19 infection and leukemogenesis in children. In this regard, the present study confirms and expands the role of Parvovirus B19 in the pathogenesis of autoimmunity and autoimmune disease in the human host [59, 60] and, in addition, confirms the possible association of B19 infection with DNA methylation signatures in childhood ALL [58].

Clearly, further intensive and extensive research efforts are necessary in order to precisely define the autoimmune crossreactions that might be involved in ALL genesis and, in general, in leukemogenesis. To this aim, Table **1** offers a clear-cut molecular platform to test sera from ALL patients for immunoreactivity against the peptides shared between B19 and human proteins associated with DNA/histone modifications.

The current study might also help design (immuno)therapeutic approaches for ALL. Notably,

Tables **1** and **2** indicate that using entire Parvovirus B19 antigens in vaccine formulations might be responsible of deleterious reactogenicity against molecules associated with DNA/histone modifications. Excessive reactogenicity has already affected previous attempts to define anti-B19 vaccines [70]. In essence, the present findings reiterate the concept that only vaccines based on peptide sequences unique to B19 [71] might guarantee effective and safe immunotherapies [72-75]. The issue appears of relevant importance given the fact the no anti-B19 vaccines is currently available [76].

AUTHORS' CONTRIBUTION

DK proposed the original idea, supervised the work, interpreted the data, and wrote the paper. AP contributed to the project definition. All authors contributed to the analyses and data discussion. All authors discussed and approved the paper.

CONFLICT OF INTERESTS

None.

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