

The Structural Basis of Germline-encoded V_H3 Immunoglobulin Binding to Staphylococcal Protein A

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Summary

The ability of human V_H3 immunoglobulins (Ig) to bind to staphylococcal protein A (SPA) via their Fab region is analogous to the binding of bacterial superantigens to T cell receptors. The present report establishes the structural basis for the interaction of SPA and V_H3 Ig. We have studied a panel of 27 human monoclonal IgM that were derived from fetal B lymphocytes. As such, these IgM were expected to be encoded by unmutated germline genes. Binding to SPA in ELISA occurred with 15 of 15 V_H3 IgM, but none of 12 IgM from the V_H1, V_H4, V_H5, or V_H6 families. The V_H sequences of the 27 IgM were derived from 20 distinct V_H elements, including 11 from the V_H3 family. Use of D, J_H, and C_L genes was similar among V_H3 and non-V_H3 IgM. A comparison of the corresponding V_H protein sequences, and those of previously studied IgM, identified a probable site for SPA binding that includes V_H3 residues in framework region 3 (FR3), and perhaps FR1 and 3' complementary determining region 2. The results thus demonstrate that among human IgM, specificity for SPA is encoded by at least 11 different V_H3 germline genes. Furthermore, like the T cell superantigens, SPA likely binds to residues in the V_H framework region, outside the classical antigen-binding site of the hypervariable loops.

Staphylococcal protein A (SPA) possesses Ig Fab-binding sites specific for determinants on the V region of the Ig H chain. These "alternative binding site(s)" of SPA are distinct from its well-characterized IgG Fc-binding sites (1-3). We previously demonstrated that the ability of Ig Fab to bind to SPA is a functional marker for Ig encoded by the largest human V_H gene family, V_H3 (4). Furthermore, SPA binding was seen with nearly all tested V_H3 IgM, and with a large portion of the tested V_H3 IgA, and V_H3 IgG F(Ab')₂ fragments (4, 5). These findings suggested that specificity for SPA is encoded by the germline sequences of many of the commonly expressed V_H3 genes. The data also implied, as did studies of SPA-binding mouse Ig (6), that the Fab site to which SPA binds involves V_H family-specific residues, most of which have been demonstrated to reside outside the conventional antigen-binding site (7-9). This association of V_H3 H chains with specificity for SPA is analogous to the ability of certain TCR V_β molecules to bind bacterial superantigens (10). Therefore, we proposed that SPA, which is a potent polyclonal activator of human B cells, be considered an Ig superantigen (5).

To further examine the structural basis for the interaction between V_H3 Ig and SPA, we have now studied the SPA-binding properties and V_H sequences of 27 monoclonal IgM derived from fetal B lymphocytes. The IgM were encoded by a spectrum of V_H elements, all of which are probably unmutated germline genes. We found that all V_H3 IgM, but

none of the other IgM, bound to SPA. Comparison of the IgM V_H sequences identified a surface containing residues of framework region 1 (FR1), 3'CDR2, and FR3, that likely has a role in SPA binding. The data also suggested that a determinant for SPA binding exists among V_H3 residues 75 to 84, in FR3. It is concluded that IgM specificity for SPA is encoded by at least 11 V_H3 germline genes, and that SPA likely binds to a V_H3 framework region, outside the classical Ag-binding site.

Materials and Methods

Monoclonal B Cell Lines. Mononuclear cells were isolated by density gradient centrifugation from second trimester fetal liver and spleen provided by the University of Washington Central Laboratory for Human Embryology (11). Fetal mononuclear cells were transformed by EBV and cloned by limiting dilution, as previously described (12). Studies described below were performed with 26 lines, randomly selected from among 62 IgM-producing cell lines (12). Beg-2, a human heterohybridoma generated from fetal spleen cells, was kindly provided by Dr. Richard Watts (University College, London, England).

Determination of IgM Concentration and L Chain Isotype. Supernatants of the 27 monoclonal IgM cell lines were assayed by ELISA in 96-well trays coated with goat F(ab')₂ anti-human F(ab')₂ (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). L chain isotype was determined by detecting bound IgM with goat F(ab')₂ anti-human κ and goat F(ab')₂ anti-human λ, both conjugated

with horseradish peroxidase (Cooper Biomedical, Malvern, PA). IgM concentration was determined by detecting bound Ig with horseradish peroxidase-conjugated goat F(ab')₂ anti-human IgM, Fc_μ-specific (Cooper Biomedical), and comparing results with a standard curve prepared with purified polyclonal human IgM (Calbiochem Novabiochem, La Jolla, CA). For subsequent binding studies, every supernatant was adjusted to ≥0.1 μg IgM/ml in tissue culture media (IMDM; Sigma Chemical Co., St. Louis, MO) that was supplemented with 10% FCS, 2 mM l-glutamine, 50 U/ml penicillin, and HAT (Boehringer Mannheim, Indianapolis, IN). All supernatants were shown to be devoid of human IgG by detection with horseradish peroxidase-conjugated Fc-specific goat F(ab')₂ anti-human IgG.

IgM Binding to SPA. IgM binding to SPA was determined by ELISA. Wells were coated with 1.5 μg SPA (Sigma Chemical Co.) in 150 μl bicarbonate buffered saline, pH 8.0, washed, incubated with 100 μl supernatant, and washed with borate buffered saline, pH 8.0, containing 0.5% Tween 20. Horseradish peroxidase-conjugated F(ab')₂ goat anti-human IgM, Fc-specific, was then incubated in the wells, followed by development with 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate (6)] (ABTS) substrate (Kirkegaard-Perry, Gaithersburg, MD). By the same protocol, each supernatant was also tested for binding to wells coated with tissue culture media supplemented with 10% FCS (negative control), and to wells coated with goat F(ab')₂ anti-human F(ab')₂ (positive control). All assays were performed in duplicate.

IgM V_H Sequence Analysis. As previously described, Ig H chain cDNA was prepared from 1 μl culture suspension, containing 1–25 monoclonal cells, with a synthetic deoxyoligonucleotide primer corresponding to the 5' region of C_μ (5'-GACGGAATTCACAGGAGAC-3') (12). The cDNA was then amplified by the PCR with the addition of a primer representing a consensus sequence in V_H codons 1 to 8 (5'-CAGGTGCAGCTGGTGAATTCTGG-3') (12). PCR product was ligated into Puc18 and sequenced by conventional methods (13, 14). Each reported sequence represents a con-

sensus of sequences from at least two independent cDNA, each of which was sequenced the entire reported length. Nucleotide mismatches occurred at a rate of one per 10,000 bp.

Results

Measurement of Binding to SPA by 27 Monoclonal IgM. A panel of 27 monoclonal IgM, each of which was expressed by a B cell line derived from fetal liver or spleen, was tested for binding to SPA in a solid phase ELISA. All 15 IgM encoded by elements from the V_H3 gene family bound to SPA (Fig. 1). In contrast, binding to SPA was not seen with any of the nine IgM from the V_H1 family, or the IgM from the V_H4, V_H5, or V_H6 families (one each) (Fig. 1). All IgM bound well to the positive control, and poorly or not at all to the negative control (data not shown). Only the V_H4 IgM (OD = 0.05) and the V_H6 IgM (OD = 0.03) bound the negative control greater than 0.01 OD units.

V_H Sequence Analysis of 27 Monoclonal IgM. The V_H nucleotide sequence of each IgM was determined. The IgM were encoded by 11 different V_H3 elements, six different V_H1 elements, and one each from the V_H4, V_H5, and V_H6 families (Fig. 1). All but five of the V_H3 and two of the V_H1 sequences were identical to known germline genes (Fig. 1). A variety of D and J_H segments was found, with a similar distribution among the V_H3 and non-V_H3 IgM (Fig. 1). The 27 J_H sequences were all ascribable to known germline genes, and none contained somatic mutations. IgM with the same V_H sequences were all clonally distinct because they differed in their D sequences, J_H sequences, or both. κ and λ L chains were equally represented among the V_H3 and the non-V_H3 IgM (Fig. 1).

Genetic Elements					IgM	Binding to Protein A (OD units)												
V _H	D _H	J _H	C _L			0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2
Family 3						[Horizontal bars representing OD units for Family 3]												
3019b9	Short	3	λ	5A10		[Horizontal bar]												
1.9III	DN1	4	λ	6H7		[Horizontal bar]												
56p1	Dir2	4	κ	5D11		[Horizontal bar]												
56p1	Dir1	2	λ	6C8		[Horizontal bar]												
56p1	Novel	4	κ	6H12		[Horizontal bar]												
26	Novel	4	κ	2D10		[Horizontal bar]												
26	Q52	4	λ	2B8		[Horizontal bar]												
26	D21/10	4	λ	2E7		[Horizontal bar]												
13-2	DN4	6	κ	4D5		[Horizontal bar]												
13-2a	Dxp4	3	λ	2B10		[Horizontal bar]												
22-2b	Short	2	κ	5D4		[Horizontal bar]												
Novel	D4	4	λ	3G11		[Horizontal bar]												
Novel	D4	4	κ	6H9		[Horizontal bar]												
Novel	Short	6	κ	2E8		[Horizontal bar]												
Novel	Short	2	κ	2E5		[Horizontal bar]												
Family 1						[Horizontal bars representing OD units for Family 1]												
51p1	Dir	2	κ	5B7		[Horizontal bar]												
51p1	DM5	4	κ	6A3		[Horizontal bar]												
2H7	Short	4	λ	6BG8		[Horizontal bar]												
2H7	D21/10	6	κ	6C9		[Horizontal bar]												
2H7	Dxp4	3	κ	2H7		[Horizontal bar]												
20p3	DFL16	4	λ	3G5		[Horizontal bar]												
20p3a	Q52	3	λ	3B2		[Horizontal bar]												
Novel	Novel	4	κ	2A12		[Horizontal bar]												
12B3	Q52	4	κ	3C9		[Horizontal bar]												
Family 5						[Horizontal bars representing OD units for Family 5]												
5-1R1	Novel	3	κ	3B8		[Horizontal bar]												
Family 4						[Horizontal bars representing OD units for Family 4]												
Beg2	Short	5	λ	Beg2		[Horizontal bar]												
Family 6						[Horizontal bars representing OD units for Family 6]												
6	Short	2	λ	6G4		[Horizontal bar]												

Figure 1. Binding to SPA by 27 fetally derived monoclonal IgM. ELISA wells were coated with SPA, washed, incubated with test IgM in supernatant at 0.1 μg/ml, washed, then incubated with horseradish peroxidase-conjugated goat F(ab')₂ anti-human IgM, followed by ABTS substrate. Binding levels are the mean of two simultaneous measurements, expressed in OD units. IgM are grouped by V_H gene family. Genetic elements identify previously reported V_H and J_H germline genes to which the obtained IgM sequences are 100% identical, and previously reported D_H germline genes to which the obtained IgM sequences are identical over at least 6 bp. Novel elements were <98% identical to reported germline genes. The germline origin of short D_H regions could not be determined. C_L isotype was determined by ELISA analysis of the respective IgM clone, which is named in the fifth column. The V_H sequences 13-2a and 20p3a differ by a single base pair from 13-2 and 20p3, respectively, but appear to be distinct germline elements because the 13-2a and 20p3a sequences were each recovered from >1 independent rearrangements (Hillson, J. L., unpublished data). References for the named genes include 56p1, 51p1, 20p3, V_H6 (15), 1.9III, 13-2, 22-2b, 5-1R1 (16), 3019b9 (17), 26c (18), 2H7 (Hillson, J. L., unpublished data), J_H elements (19), and D_H elements (8, 19-24).

	10	20	30	40	50	60	70	80	90	110	
FAMILY 3 Binders			<u>CDR1</u>		<u>CDR2</u>				<u>(D/N)</u>	<u>CDR3</u>	<u>(J)</u>
3 - 5A10	-GGVQPGRSLRLSCAASGTFSS	YGMHWVROAPGKLEWVAIVHY	DGSNKYYADSVGRFTISRDNSKNTLYLQMNLSRAEDTAVYYC	ARDHC	-----	AFDIWGQGMVTVSS					
3 - 6H7	-----	-----	-----	-----	-----	-----	-----	-----	KDFLSSRAL	-----	Y.....L.....
3 - 5D11	-----	-----	-----	-----	-----	-----	-----	-----	EVGGSSG	-----	Y.....L.....
3 - 6C8	-----	-----	-----	-----	-----	-----	-----	-----	DRRGA	-----	WY..L..R..L.....
3 - 6H12	-----	-----	-----	-----	-----	-----	-----	-----	DFIDA	-----	Y.....L.....
3 - 2D10	-----	-----	-----	-----	-----	-----	-----	-----	KDARDVY	-----	DY..Y..L.....
3 - 2B8	-----	-----	-----	-----	-----	-----	-----	-----	KDVGEG	-----	Y.....L.....
3 - 2E7	-----	-----	-----	-----	-----	-----	-----	-----	KVGGGFS	-----	Y.....L.....
3 - 4D5	-----	-----	-----	-----	-----	-----	-----	-----	AARVAAGPYQLYYTYGM	-----	V.....T.....
3 - 2B10	-----	-----	-----	-----	-----	-----	-----	-----	AVA	-----	-----
3 - 5D4	-----	-----	-----	-----	-----	-----	-----	-----	DPIN	-----	WY..L..R..L.....
3 - 3G11	-----	-----	-----	-----	-----	-----	-----	-----	VFTSC	-----	Y.....L.....
3 - 6H9	-----	-----	-----	-----	-----	-----	-----	-----	DLIIMP	-----	Y.....L.....
3 - 2E8	-----	-----	-----	-----	-----	-----	-----	-----	ED	-----	YYYGM.V.....T.....
3 - 2E5	-----	-----	-----	-----	-----	-----	-----	-----	GYH	-----	WY..L..R..L.....
3* - POM	-----	-----	-----	-----	-----	-----	-----	-----	DAGPYVSPITF	-----	AHY..L.....
3* - LAY	-----	-----	-----	-----	-----	-----	-----	-----	DAGPYVSPITF	-----	AH.....L.....
3* - KL1	-----	-----	-----	-----	-----	-----	-----	-----	KLRSGLVPYY	-----	S.....L..N.....
3* - RIV	-----	-----	-----	-----	-----	-----	-----	-----	KLSTAASGFTFDI	-----	YGM.V.....T.....
FAMILY 3 Non-binders											
3* - SJI	-----	-----	-----	-----	-----	-----	-----	-----	KGVCSSSSCYSYYYYYYMDVW	-----	K..T.....
3* - TS2	-----	-----	-----	-----	-----	-----	-----	-----	DRVAAYASVFFIDS	-----	G.....
FAMILIES 1,5,4,6 Non-binders											
1 - 5B7	-AE.KK..S.VKW..K..G..TG--	AIS.....Q...	MGG.IP-IPGTAN..QKFK	.V..TA.E.TS.A.MELS	..S.....	LRWGS	-----	WY..L..R..L.....			
1 - 6A3	-AE.KK..S.VKW..K..G..TG--	AIS.....Q...	MGG.IP-IPGTAN..QKFK	.V..TA.E.TS.A.MELS	..S.....	LTGL	-----	Y.....L.....			
1 - 6B8	-AE.KK..A.VKV..K..Y..T--	A.....Q...	MGW.NA-GNG.TK..QKFK	.V..T.T.AS.A.MELS	..S.....	E	-----	Y.....L.....			
1 - 6C9	-AE.KK..A.VKV..K..Y..T--	A.....Q...	MGW.NA-GNG.TK..QKFK	.V..T.T.AS.A.MELS	..S.....	VTLGGIKF	-----	YYYGM.V.....T.....			
1 - 2H7	-AE.KK..A.VKV..K..Y..T--	A.....Q...	MGW.NA-GNG.TK..QKFK	.V..T.T.AS.A.MELS	..S.....	VDPWGSYY	-----	Y.....L.....			
1 - 3G5	-AE.KK..A.VKV..K..Y..TG--	Y.....Q...	MGW.NA-NSGGIN..QKFK	.V.MT.T.IS.A.MELSR	..S.....	SLYGYV	-----	D.....L.....			
1 - 3B2	-AE.KK..A.VKV..K..Y..TG--	Y.....Q...	MGR.NA-NSGGIN..QKFK	.V.MT.T.IS.A.MELSR	..S.....	DLTG	-----	D.....L.....			
1 - 2A12	-AE.KK..A.VKV..K..Y..T--	DIN...T.Q...	MGR.NA-NNG.TG..QKFK	.V.MT.NT.IS.A.MELS	..S.....	GKGGE	-----	L.....			
1 - 3C9	-AE.KK..S.VKV..K..G..AIS--	Q.....Q...	MGR.IP-ILGLAN..QKFK	.V..TA.K.TS.A.MELS	..S.....	WINWGS	-----	Y.....L.....			
5 - 3B6	-AE.KK..E..KI..KG..YS.T--	WIG...M.....	MGT.YP-GD.DTRTSP.LQ.QV	.A.K.IS.A..WS..K.S..M..	-----	RRV	-----	D.....			
4 - BEG2	-P.L.K.SQT.S.T.TV..GSI..GS.YMN.I..PG	-----	IGR.Y--TSGSIN.NP.L.S.V..V.T..QFS..L.VTP	-----	-----	QS	-----	NW..P.....L.....			
6 - 6G4	-P.L.K.SQT.S.T.T.I..DSV..NSAAMN.I..S.SR	-----	LGRTY.RSKWYND.V...S.I..NP.T...QFS..L.VTP	-----	-----	G	-----	WY..L..R..L.....			
Comparisons:	Xx x	xXx x X	X	x	X	xxx X x X xx X xxX x					

Figure 2. Protein sequences of the H chain V regions of monoclonal IgM. Amino acids were translated from the obtained nucleotide sequences of 27 IgM described in Fig. 1, and are shown from codon 9 through codon 114. Sequences are grouped by V_H gene family and ability to bind to protein A. For comparison, the V_H sequences of six previously reported V_{H3} IgM are shown at the bottom of the V_{H3} group, with their names italicized and marked with an asterisk (4, 25–28). (Top) Sequence from clone 5A10. Amino acids of other sequences are specified only where different from 5A10. (–) Relative deletion. (Bottom) (X) Position at which a single amino acid residue is identical among and unique to all our V_{H3} sequences. (x) All V_{H3} residues at that position are identical or conserved, and also present in the V_{H4}, V_{H5}, or V_{H6} sequence, but not the V_{H1} sequences. The sequences of the previously reported sequences (Pom, Lay, Riv, KL1, SJI, and TS2) are excluded from the analysis leading to the assignment of X and x designators. Sequence organization and CDR are according to Kabat et al. (8). The nucleotide sequences from which these amino acid sequences were generated are available from EMBL/GenBank/DDJB among the sequences having the accession numbers L04323–L04346 and L03815–L03830.

Each of the 20 different V_H nucleotide sequences encoded a different translated protein sequence, i.e., six different proteins from the V_{H1} family, 11 from V_{H3}, and one each from V_{H4}, V_{H5}, and V_{H6} (Fig. 2). Comparison of these protein sequences identified 23 positions at which the amino acid residue is invariant among the V_{H3} sequences and different from the residue(s) present in the V_{H1} sequences. At position 82a also, the V_{H3} residues are identical, and different from the V_{H1} residues, except for a conservative substitution in one V_{H3} sequence, 3G11 (Fig. 2).

Discussion

We have determined the SPA-binding ability and the V_H sequences of a panel of 27 monoclonal human IgM derived from fetal B lymphocytes. We found that 15 of 15 V_{H3} IgM bound to SPA, whereas none of the IgM from the V_{H1}, V_{H4}, V_{H5}, and V_{H6} families bound to SPA. No correlation was seen between SPA binding and D or J_H gene use, or C_L isotype. This result confirms that binding to SPA is a functional marker for V_{H3} IgM, and indicates that SPA-binding IgM are encoded by many of the V_{H3} genes expressed in the human fetal repertoire.

The fetal origin of the studied IgM makes it likely that the V_H sequences they contained were encoded by unmutated genes, and were not subject to selection by exogenous Ag. In fact, 12 of the distinct V_H sequences, including six from the V_{H3} family, were identical to those of known V_H germline genes. The finding that all IgM had unmutated J_H sequences provides additional evidence that the novel V_H sequences, four V_{H3} and one V_{H1}, are probably unmutated, and therefore identical to V_H germline sequences that have not yet been reported. Thus, the data clearly identify 11 distinct V_{H3} genes that encode SPA-binding IgM. Furthermore, specificity for SPA was encoded by the unmutated form of at least six, and probably all 11, of these V_{H3} germline genes.

The haploid genome is estimated to contain between 25 and 50 V_{H3} germline gene loci, up to two thirds of which are functional (16, 29). Thus, about 20–40% of V_{H3} germline loci, and a larger portion of functional V_{H3} germline loci, have now been directly demonstrated to encode SPA-binding Ig. It seems likely that many of the remaining V_{H3} loci will also encode SPA-binding proteins. In previous studies of polyclonal Ig purified from blood, a small minority of total V_{H3} IgM, and a larger subset of total V_{H3} IgA and V_{H3} IgG

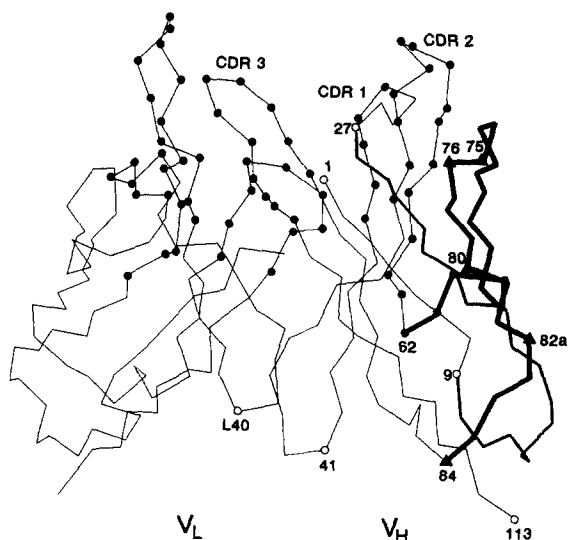


Figure 3. V_H3 residues implicated in the Fab site that binds to SPA. Shown is an alpha carbon backbone model of a V_L - V_H pair from a V_H3 Ig. (Right) V_H polypeptide; (left) V_L . (Filled dots) V_H and V_L CDR residues. V_H CDR loops are labeled. V_H regions containing residues associated with binding to SPA are indicated by a medium line in FR1 (9-27) and a heavy line in 3' CDR2 (62-65) and FR3 (66-84). (Filled triangles) V_H FR3 residues at which a nonconservative substitution is associated with loss of specificity for SPA. Also indicated (open circles) are the first and terminal V_H residues (1 and 113), and a FR2 residue on the V_H (41) and V_L (L40) chain. The figure is modified from that of McPC603 in Kabat et al. (8).

did not demonstrate Fab-mediated binding to SPA (4, 5). The elements encoding these SPA-nonbinding V_H3 Ig could have lost specificity for SPA through somatic modification, or they might belong to a subset of V_H3 germline genes that encode SPA-nonbinding proteins. It is also possible that certain combinations of D , J_H , V_L , and J_L genes abrogate V_H3 binding to SPA. However, the broad variety of these genes (D , J_H , V_L , J_L) that have been found to encode SPA-binding IgM argues that this effect occurs infrequently, if at all (4, 28, 30).

The site on Ig Fab that binds to SPA has been localized to the variable region of the H chain (31), and shown to be functionally distinct from a conventional hapten-binding site (32). Our data now provide structural evidence that SPA binds outside the classical antibody binding site. The V_H sequences from our IgM identify 24 amino acid positions at which all V_H3 sequences have a conserved residue, and all V_H1 sequences differ by a nonconservative change. Two of these positions are in FR2, which is inaccessible to solvent (8). The remaining 22 positions localize a candidate binding site for SPA to two peptides, one in FR1 (residues 9-27), the other in 3' CDR2/FR3 (residues 62-84). Seven of these positions, indicated at the bottom of Fig. 2 (X), are strongly associated with IgM ability to bind to SPA, because their V_H3 residues are unique. The other 15 positions, (x), could also have a role in SPA binding, even though their V_H3 amino acids appear in the V_H6 sequence (13 of 15 positions), the V_H4 se-

quence (12 positions), or the V_H5 sequence (5 positions). In an intact, folded Ig molecule, the two peptides reside in closely adjacent, solvent-exposed, β -pleated sheets that define a region on the lateral aspect of the Fab molecule that is removed from the V_H hypervariable loops (8) (Fig. 3). This V_H structure is structurally analogous to the region bound by T cell superantigens on the β chain of TCRs (33, 34).

Further insight into the protein A-binding site of V_H3 Ig can be gained by examining six previously reported V_H sequences, from IgM Pom, Lay, Riv, KL1, SJ1, and TS2 (Fig. 2) (25-27). IgM Pom, Lay, Riv, and KL1 bind to SPA (4, 28). In contrast, IgM SJ1 and TS2 have been reported to not bind to SPA (28), even though their protein sequences are 94 and 96% identical, respectively, to those encoded by 1.9III and 56p1, which we found to bind to SPA (Fig. 1). A sequence comparison that includes SJ1 and TS2 identifies five FR3 positions, 75, 76, 80, 82a, and 84, at which nonconservative substitutions in a V_H3 sequence are associated with inability to bind protein A (Fig. 2). Each of these positions was also identified by the above analysis of our V_H3 sequences (Fig. 2). Thus, some of these substitutions could have abrogated SPA binding. The other substitutions in SJ1 and TS2 are unlikely to have abrogated SPA binding because, either (a) they resulted in a conservative amino acid change (residues 28 in FR1, and 59 in CDR2); (b) they occurred at a site that is inaccessible to SPA (residue 40 in FR2); or (c) in other V_H3 IgM, nonconservative substitutions at that position were associated with retained ability to bind SPA (residues 52, 52a, and 57 in CDR2) (Fig. 2).

These findings suggest that the FR3 75-84 peptide contains a determinant that is critical for binding to SPA. Residues 75-84 begin in a FR3 loop, and extend to include a portion of FR3 that is nearer to the C region than to the CDR (Fig. 3). Among residues 75-84, nonconservative substitutions that do not abrogate SPA binding are found in IgM KL1 (T to P, residue 77); Lay (R to Q, residue 83); and Pom (Q to I and R to Q, residues 81 and 83) (Fig. 2). It seems likely, therefore, that only a limited number of residues in the 75 to 84 peptide is directly involved in the SPA- V_H3 interaction. This prediction can be directly tested by site-directed mutagenesis.

In conclusion, we have studied the SPA-binding properties of a panel of monoclonal human IgM produced by B lymphocytes derived from fetal liver or spleen. Binding occurred with all V_H3 molecules, but no others, indicating that specificity for SPA is encoded by at least 11 different V_H3 germline genes. Analysis of IgM V_H sequences indicated that conserved residues in V_H3 FR1 and 3' CDR2/FR3 likely play a role in SPA recognition by V_H3 proteins. Furthermore, the site to which SPA binds might directly involve residues in the FR3 75-84 peptide. These findings elucidate the structural basis of the nonclassical binding specificity that V_H3 Ig have for SPA, and demonstrate a structural analogy between the SPA-Fab interaction, and the binding of bacterial superantigens to TCRs.

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