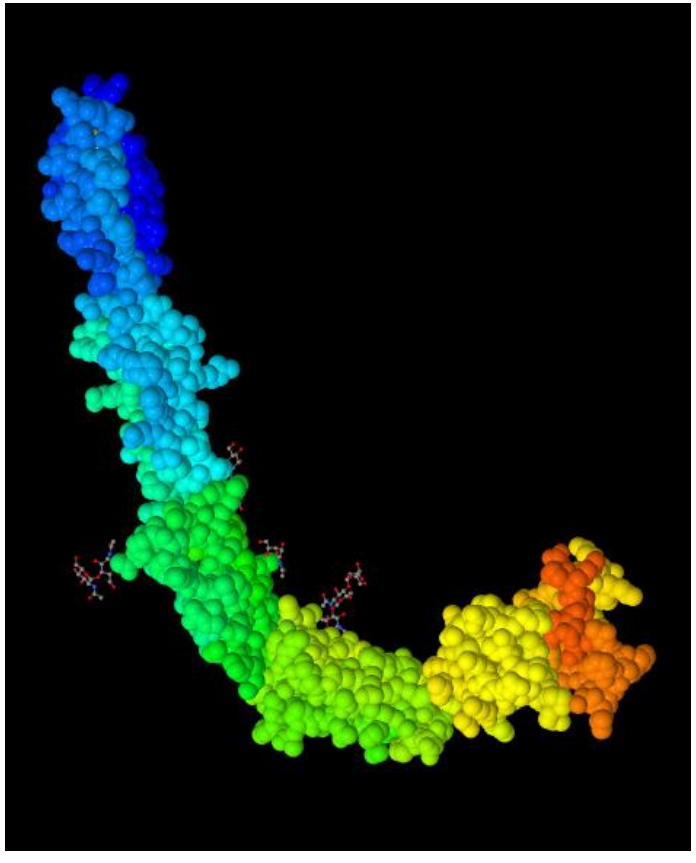
Anti-b2glycoprotein I antibodies of the IgA isotype recognize epitopes in domain 3, 4 and 5 that are located in the same lateral zone of the molecule

Running tittle: IgA anti-B2GP1 recognizes epitopes in domains 3, 4 and 5.

Manuel Serrano (Spain)

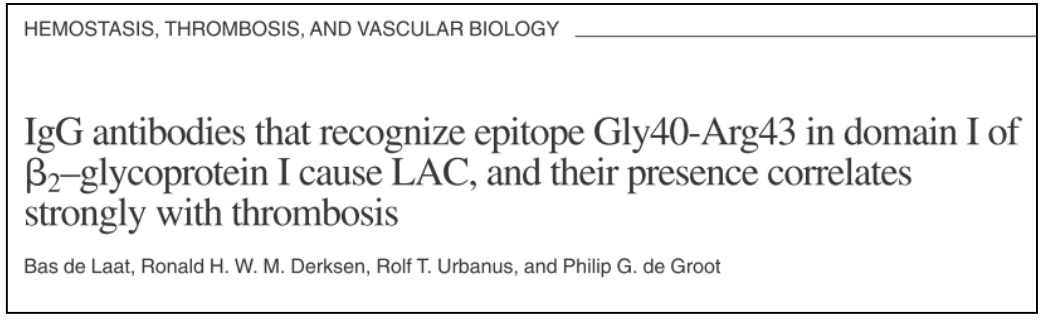
B2 glycoprotein I



A protein of 321 amino acids, distributed in 5 sushi domains

It is elaborated by liver, heart, kidney and bowel

Several conformations are described, the most important are, circular and fish hook



HEMOSTASIS, THROMBOSIS, AND VASCULAR BIOLOGY

IgG antibodies that recognize epitope Gly40-Arg43 in domain I of β_2–glycoprotein I cause LAC, and their presence correlates strongly with thrombosis

Bas de Laat, Ronald H. W. M. Derksen, Rolf T. Urbanus, and Philip G. de Groot

Lupus (2016) 25, 905–910
http://lup.sagepub.com

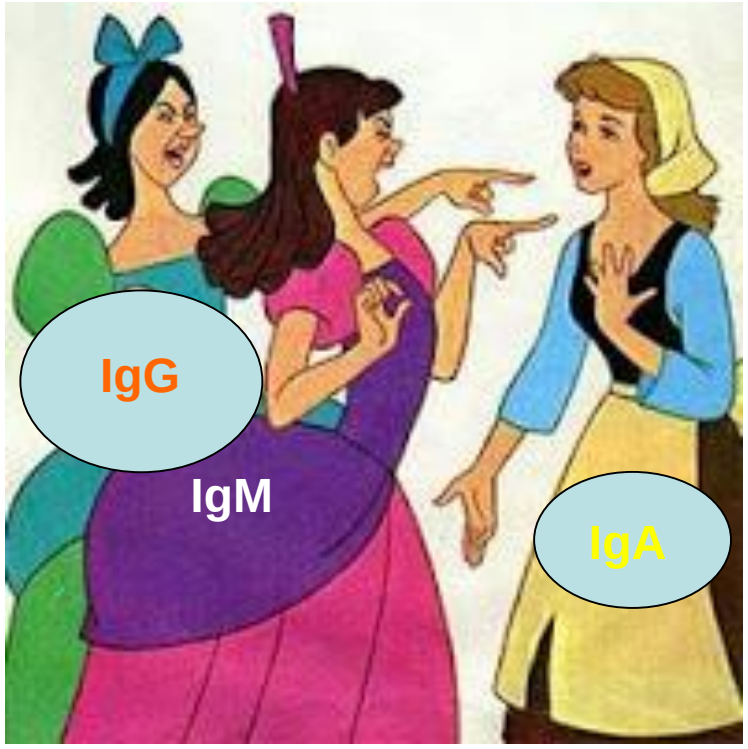
SPECIAL ARTICLE

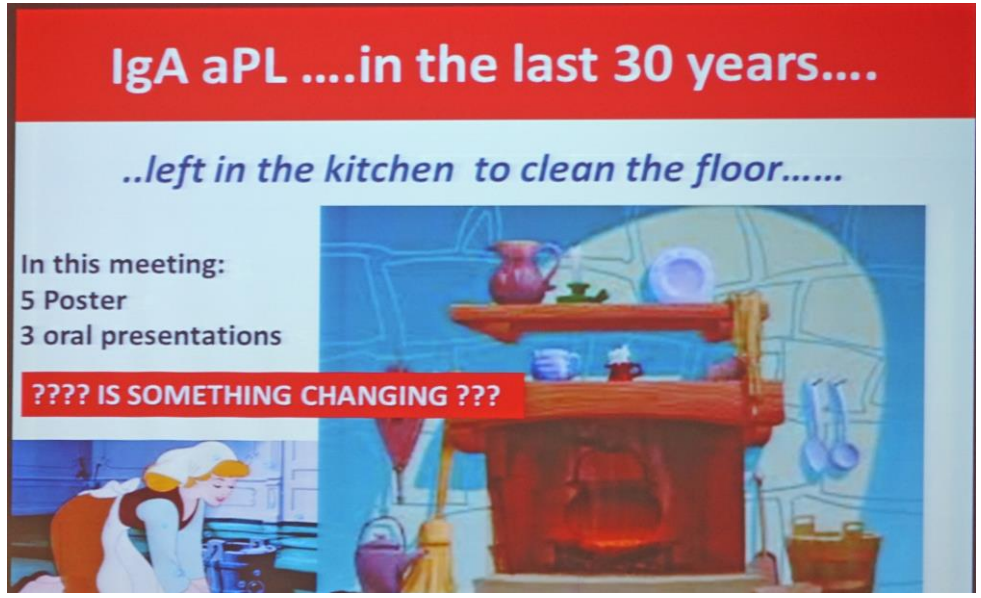
Anti-beta-2 glycoprotein I epitope specificity: from experimental models to diagnostic tools

PL Meroni
Department of Clinical Sciences and Community Health, University of Milan, Laboratory of Immuno-rheumatology Research, Istituto Auxologico Italiano, Milan, Italy

-aB2GP1 consensus antibodies IgG/IgM can recognize every protein domains

-Traditionally epitopes located in **Domain I** have been related to thrombotic events





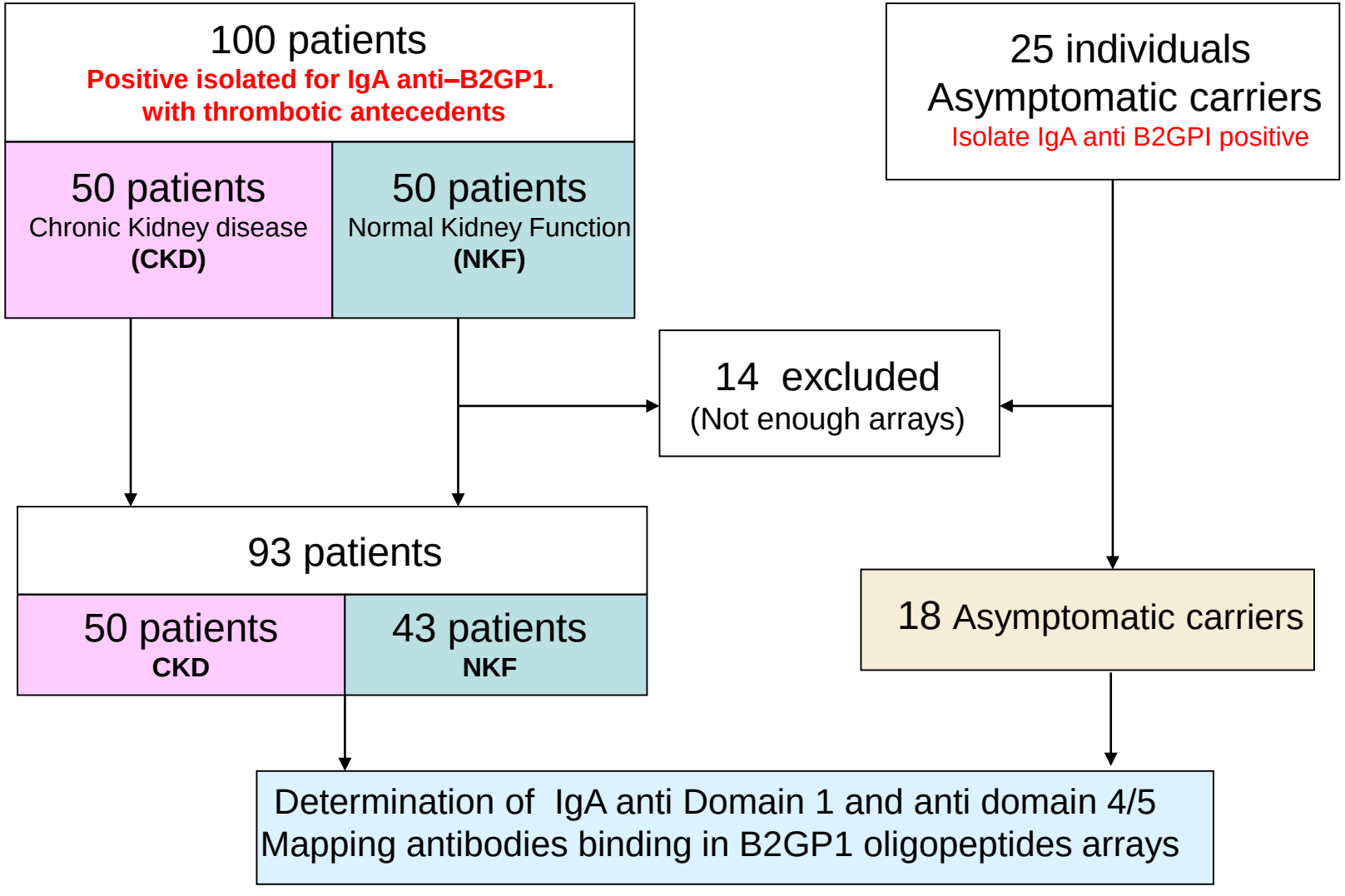
Slade from Dr. Angela Tincani

IgA aB2GPI are not included in classification criteria, but since Galveston 2010, task force recommend its determination in case of suspection of seronegative APS

Controversy and IgA isotype: Why?

- Populations selected for study IgA isotype
- Heterogeinity in the different laboratory methods
- Its clinical relevance is only showed if presents in isolated form
- No Recognition of domain I B2GP1

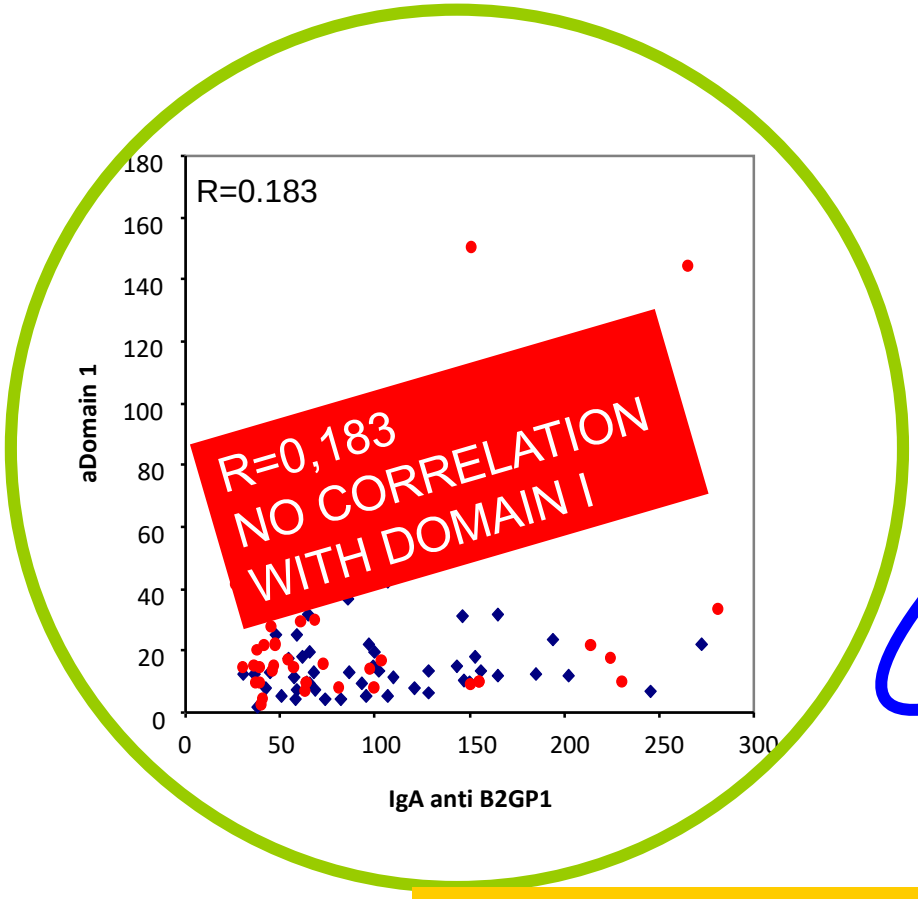
What exactly recognizes IgA aB2GP1 antibodies?

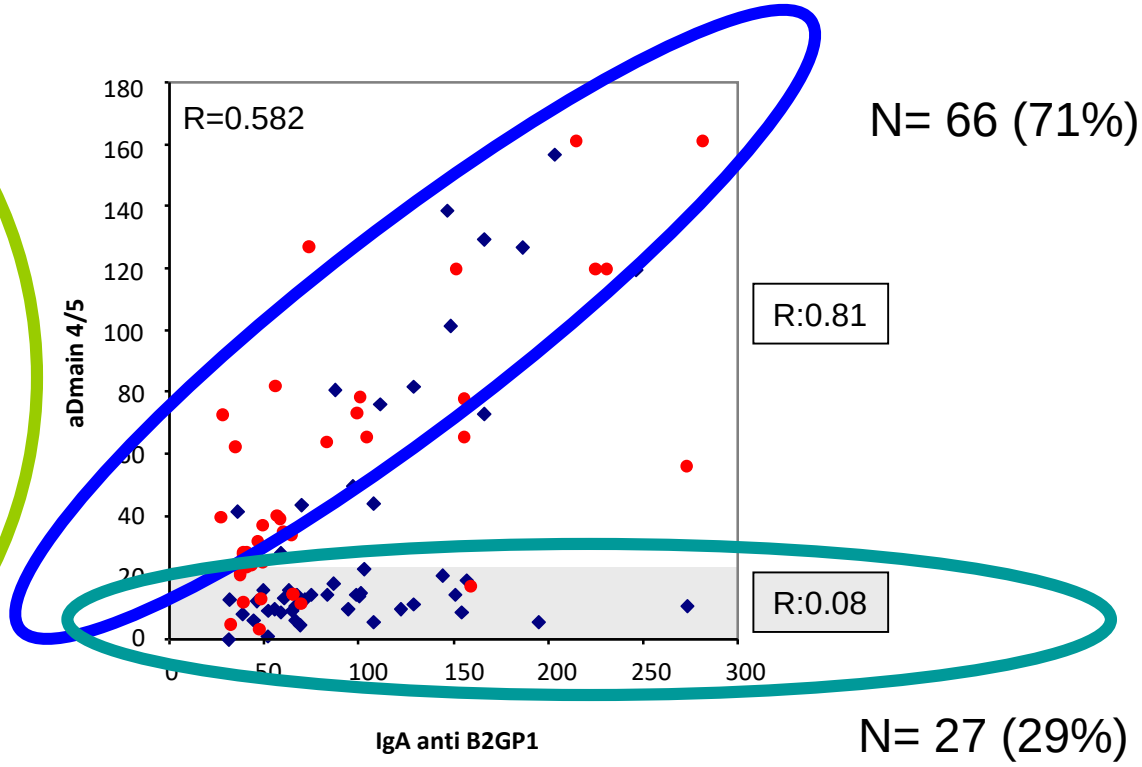

100 patients
Positive isolated for IgA anti–B2GP1.
with thrombotic antecedents
50 patients
Chronic Kidney disease
(CKD)
50 patients
Normal Kidney Function
(NKF)
25 individuals
Asymptomatic carriers
Isolate IgA anti B2GPI positive
14 excluded
(Not enough arrays)
93 patients
50 patients
CKD
43 patients
NKF
18 Asymptomatic carriers
Determination of IgA anti Domain 1 and anti domain 4/5
Mapping antibodies binding in B2GP1 oligopeptides arrays

APS events CKD vs NKF patients

APS event	Global		CKD (N=50)		NKF (N=43)		P
Deep venous thrombosis	58	(32,3%)	31	(62%)	27	(62,8%)	0,938
Arterial thrombosis	15	(16,1%)	9	(18%)	6	(14%)	0,597
Pulmonary embolism	16	(17,2%)	1	(2%)	15	(34,9%)	<0.001
Stroke	6	(6,5%)	1	(2%)	5	(11,6%)	0.092
Myocardial infarction	11	(11,8%)	5	(10%)	6	(14%)	0,556
Graft Thrombosis (kidney transplanted patients	14	(15%)	14	(28%)	-	-	-
Vascular access trombosis (dialized patients)	*22*	(23,7%)	22	(44%)	-	-	-

Correlation ELISA Domain I and Domain IV/V vs ELISA complete protein





N= 66 (71%)

N= 27 (29%)

50% of patients were positive for D IV/V

Comparison populations positive vs negative for ELISA domains I and IV/V

Characteristic	aD1 negative	a D1-positive	
	Number / mean	Number / mean	p value
Age (years)	57,1 ±2.0	60,2 ±3,3	0,434
Sex (men)			
Deep venous			
Arterial thrombosis	12 (16,4%)	3 (15%)	0,877
Pulmonary embolism	12 (16,4%)	4 (20%)	0,709
Stroke	3 (4,1%)	3 (15%)	0,079
Myocardial infarction	8 (11%)	3 (15%)	0,620
Graft loss by thrombosis	9 (12,3%)	5 (25%)	0,160
Vascular access trombosis	20 (27,4%)	2 (10%)	0,105

IgA aB2GP1 antibodies must recognize another domains

Characteristic	aD4/5 negative	aD4/5-positive	
	Number / mean	Number / mean	p value
Age (years)	56,8 ±2,3	58,9 ±2,5	0,54
Sex (men)			0,936
Deep venous			0,689
Arterial thrombosis	8 (17,8%)	7 (14,6%)	0,676
Pulmonary embolism	7 (15,6%)	9 (18,8%)	0,683
Stroke	2 (4,4%)	4 (8,3%)	0,446
Myocardial infarction	5 (11,1%)	6 (12,5%)	0,836
Graft loss by thrombosis	9 (20%)	5 (10,4%)	0,197
Vascular access trombosis	14 (31,1%)	8 (16,7%)	0,101

No clinical differences were found between positives and negatives

Array design 1

Map of domains and peptides of B2 Glycoprotein 1

Peptide Number	Sequence	Position	Domain	Peptide Number	Sequence	Position	Domain	Peptide Number	Sequence	Position	Domain
1	GRTCPKPDDLPFSTV	1-15	1	28	GKWSPELPVCAPIIC	109-123	2-3	55	DGYSLDGPEEIECTK	217-231	4
2	PKPDDLPFSTVVPLK	5-19	1	29	PELPVCAPIICPPPS	113-127	2-3	56	LDGPEEIECTKLGNW	221-235	4
3	DLPFSTVVPLKTFYE	9-23	1	30	VCAPIICPPPSIPTF	117-131	2-3	57	EEIECTKLGNWSAMP	225-239	4
4	STVVPLKTFYEPGEE	13-27	1	31	IICPPPSIPTFATLR	121-135	3	58	CTKLGNWSAMPSCKA	229-243	4
5	PLKTFYEPGEEITYS	17-31	1	32	PPSIPTFATLRVYKP	125-139	3	59	GNWSAMPSCKASCKV	233-247	4-5
6	FYEPGEEITYSCKPG	21-35	1	33	PTFATLRVYKPSAGN	129-143	3	60	AMPSCKASCKVPVKK	237-251	4-5
7	GEEITYSCKPGYVSR	25-39	1	34	TLRVYKPSAGNNSLY	133-147	3	61	CKASCKVPVKKATVV	241-255	4-5
8	TYSCKPGYVSRGGMR	29-43	1	35	YKPSAGNNSLYRDTA	137-151	3	62	CKVPVKKATVVYQGE	245-259	5
9	KPGYVSRGGMRKFIC	33-47	1	36	AGNNSLYRDTAVFEC	141-155	3	63	VKKATVVYQGERVKI	249-263	5
10	VSRGGMRKFICPLTG	37-51	1	37	SLYRDTAVFECLPQH	145-159	3	64	TVVYQGERVKIQEKF	253-267	5
11	GMRKFICPLTGLWPI	41-55	1	38	DTAVFECLPQHAMFG	149-163	3	65	QGERVKIQEKFKNGM	257-271	5
12	FICPLTGLWPINTLK	45-59	1	39	FECLPQHAMFGNDTI	153-167	3	66	VKIQEKFKNGMLHGD	261-275	5
13	LTGLWPINTLKCTPR	49-63	1-2	40	PQHAMFGNDTITCTT	157-171	3	67	EKFKNGMLHGDKVSF	265-279	5
14	WPINTLKCTPRVCPF	53-67	1-2	41	MFGNDTITCTTHGNW	161-175	3	68	NGMLHGDKVSFFCKN	269-283	5
15	TLKCTPRVCPFAGIL	57-71	1-2	42	DTITCTTHGNWTKLP	165-179	3	69	HGDKVSFFCKNKEKK	273-287	5
16	TPRVCPFAGILENGA	61-75	1-2	43	CTTHGNWTKLPECRE	169-183	3	70	VSFFCKNKEKKCSYT	277-291	5
17	CPFAGILENGAVRYT	65-79	2	44	GNWTKLPECREVKCP	173-187	3-4	71	CKNKEKKCSYTEDAQ	281-295	5
18	GILENGAVRYTTFEY	69-83	2	45	KLPECREVKCPFPSR	177-191	3-4	72	EKKCSYTEDAQCIDG	285-299	5
19	NGAVRYTTFEYPNTI	73-87	2	46	CREVKCPFPSRPDNG	181-195	3-4	73	SYTEDAQCIDGTIEV	289-303	5
20	RYTTFEYPNTISFSC	77-91	2	47	KCPFPSRPDNGFVNY	185-199	4	74	DAQCIDGTIEVPKCF	293-307	5
21	FEYPNTISFSCNTGF	81-95	2	48	PSRPDNGFVNYPAKP	189-203	4	75	IDGTIEVPKCFKEHS	297-311	5
22	NTISFSCNTGFYLNG	85-99	2	49	DNGFVNYPAKPTLYY	193-207	4	76	IEVPKCFKEHSSLAF	301-315	5
23	FSCNTGFYLNGADSA	89-103	2	50	VNYPAKPTLYYKDKA	197-211	4	77	KCFKEHSSLAFWKTD	305-319	5
24	TGFYLNGADSAKCTE	93-107	2	51	AKPTLYYKDKATFGC	201-215	4	78	EHSSLAFWKTDASDV	309-323	5
25	LNGADSAKCTEEGKW	97-111	2	52	LYYKDKATFGCHDGY	205-219	4	79	SLAFWKTDASDVKPC	312-326	5
26	DSAKCTEEGKWSPEL	101-115	2	53	DKATFGCHDGYSLDG	209-223	4				
27	CTEEGKWSPELPVCA	105-119	2	54	FGCHDGYSLDGPEEI	213-227	4				

Color code

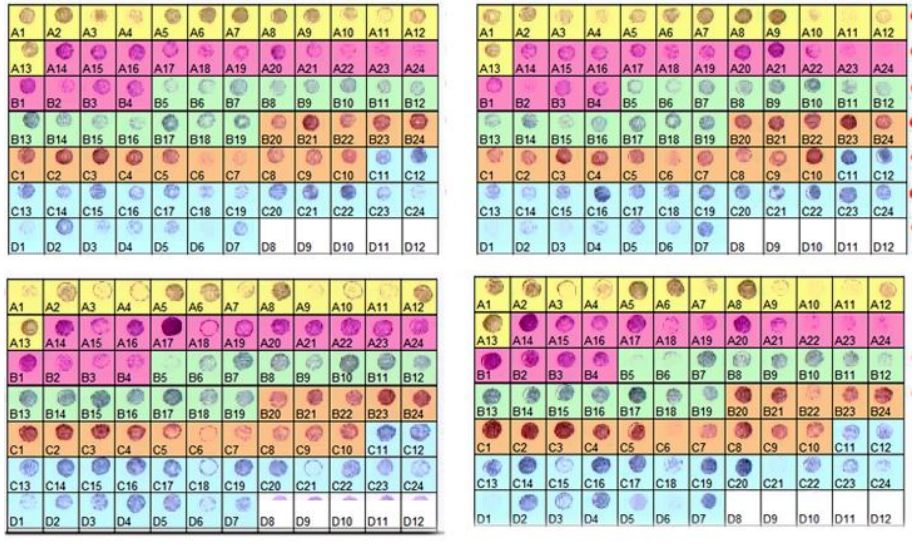
Domain 1
Domain 2
Domain 3
Domain 4
Domain 5

- Each array contains 79 peptides of 15-aa
- Each peptide has overlapping of 4 amino acids with the previous one and with the next one

Array design 2

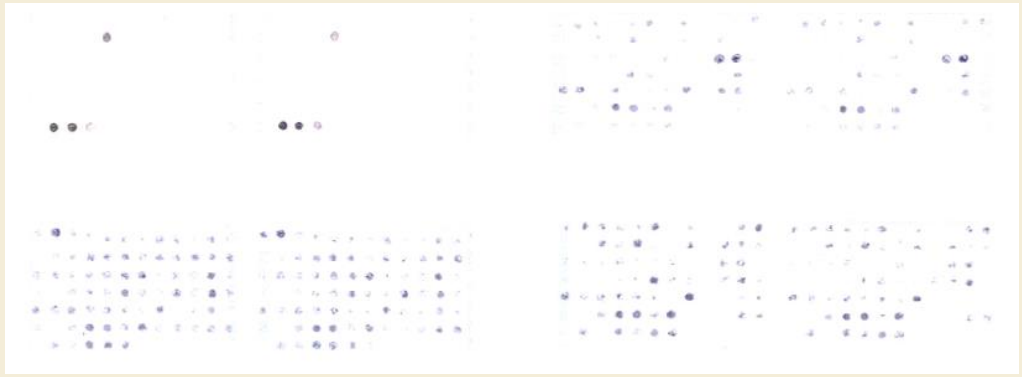
NP_000033

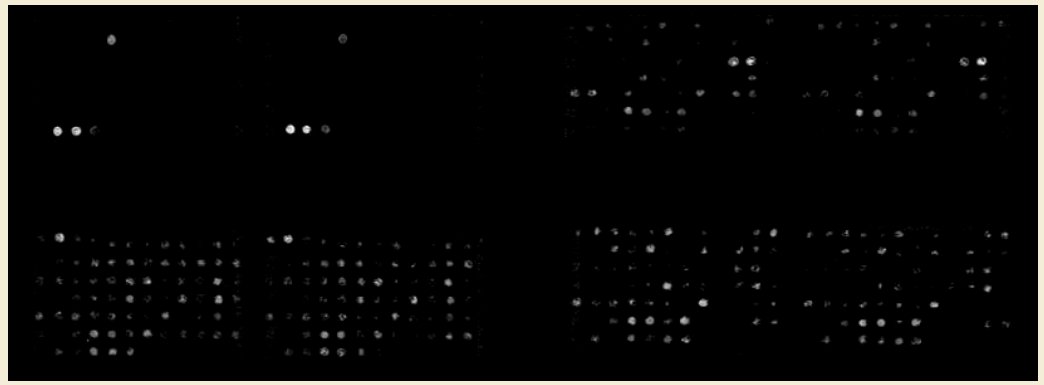
Anti D IV/V Monoclonal Ab



Examples of patients

Examples, policlonal response, with innespecific recognition, aparently there are not a common patron





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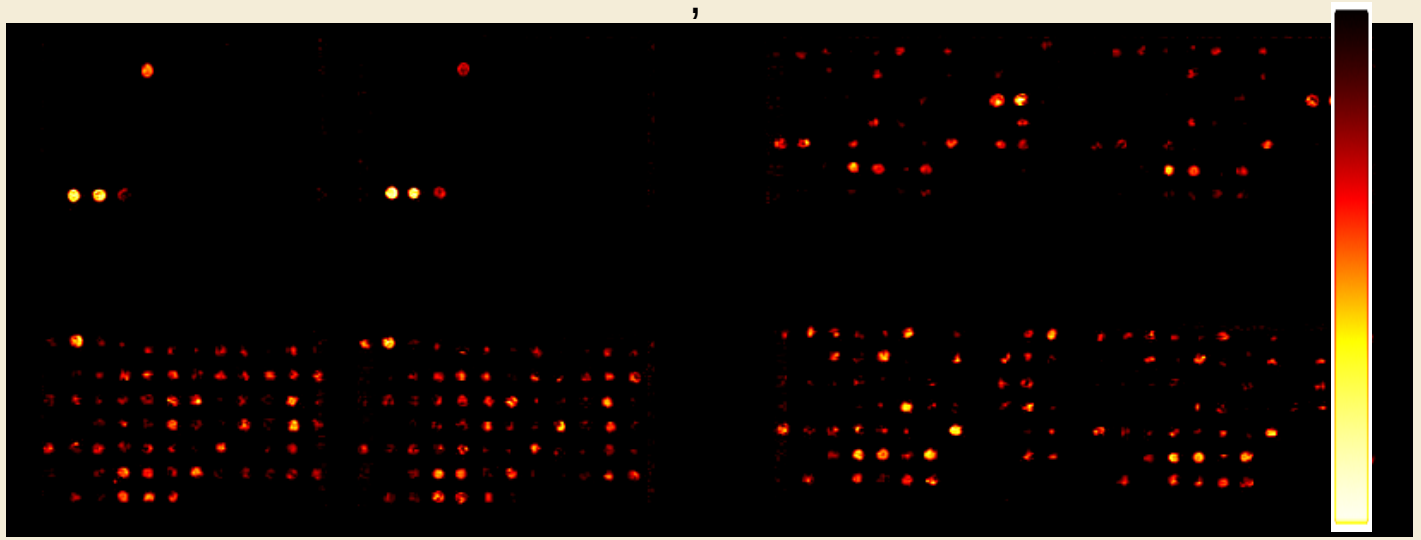
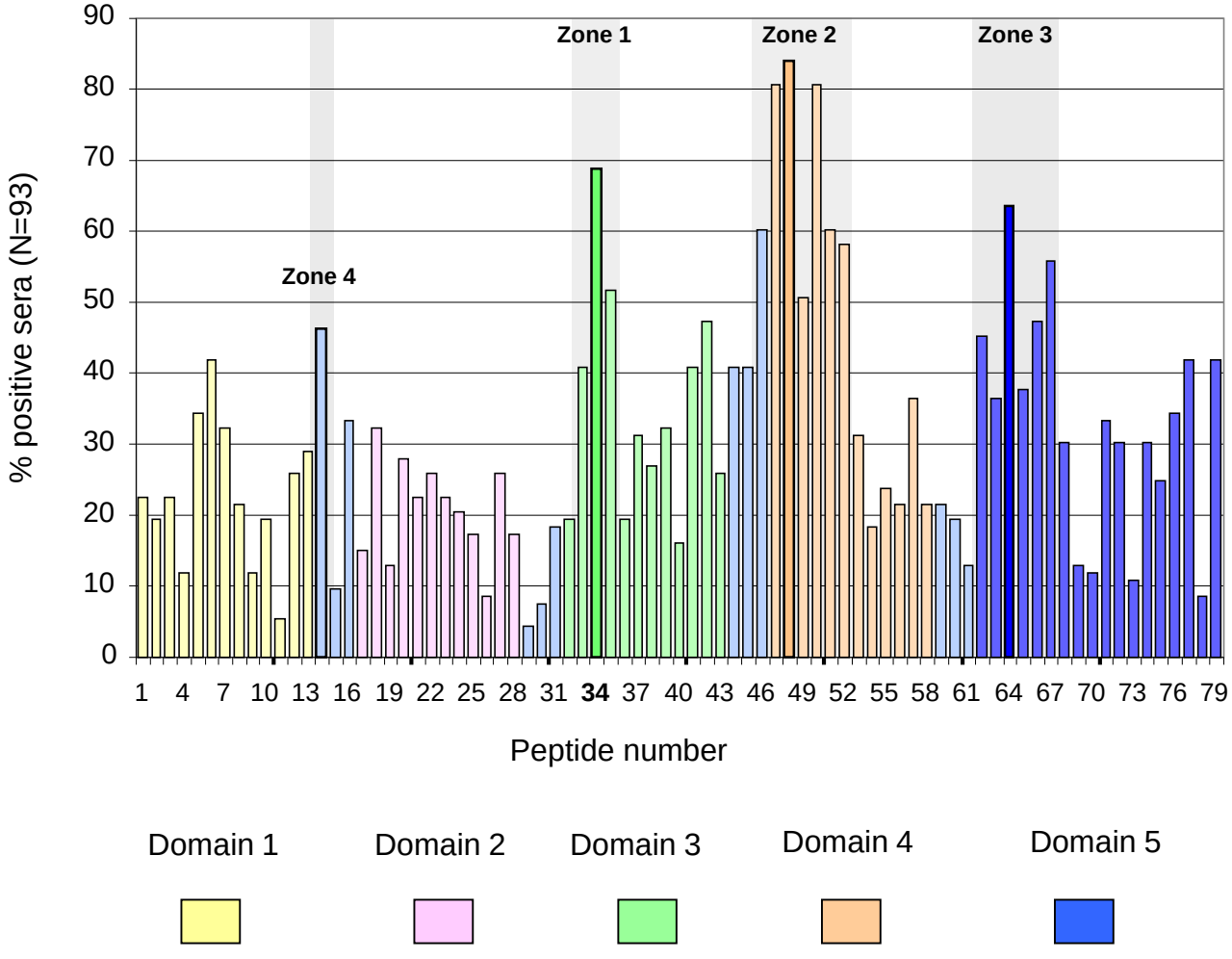


Peptides recognition patterns of patients

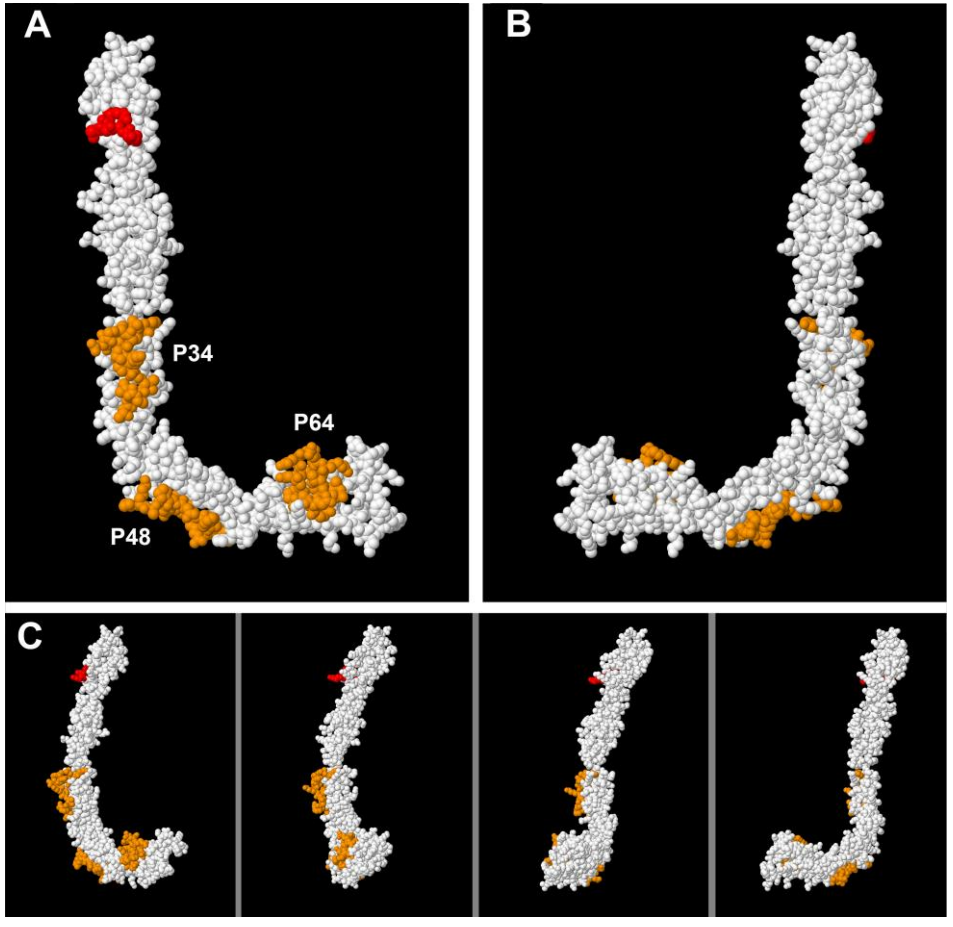


Three peptides with high antigenicity

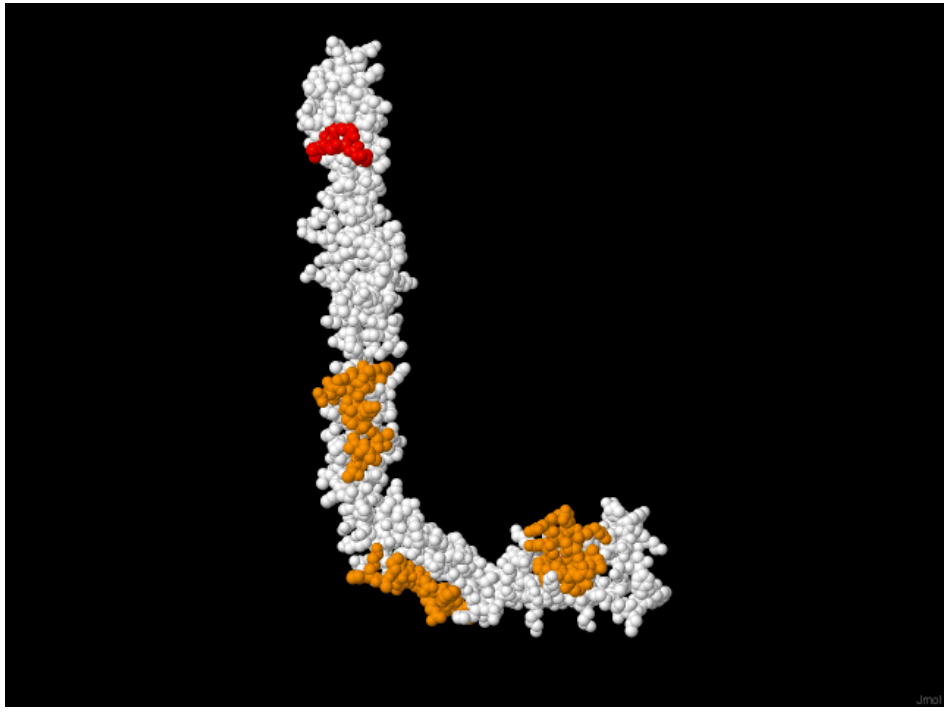
-Zone 1 **P34** (peptides 33-35) Domain 3
-Zone 2 **P48** (peptides 46-52) Domain 4
-Zone 3 **P64** (peptides 62-67). Domain 5
-Zone 4 P13 Domain 1

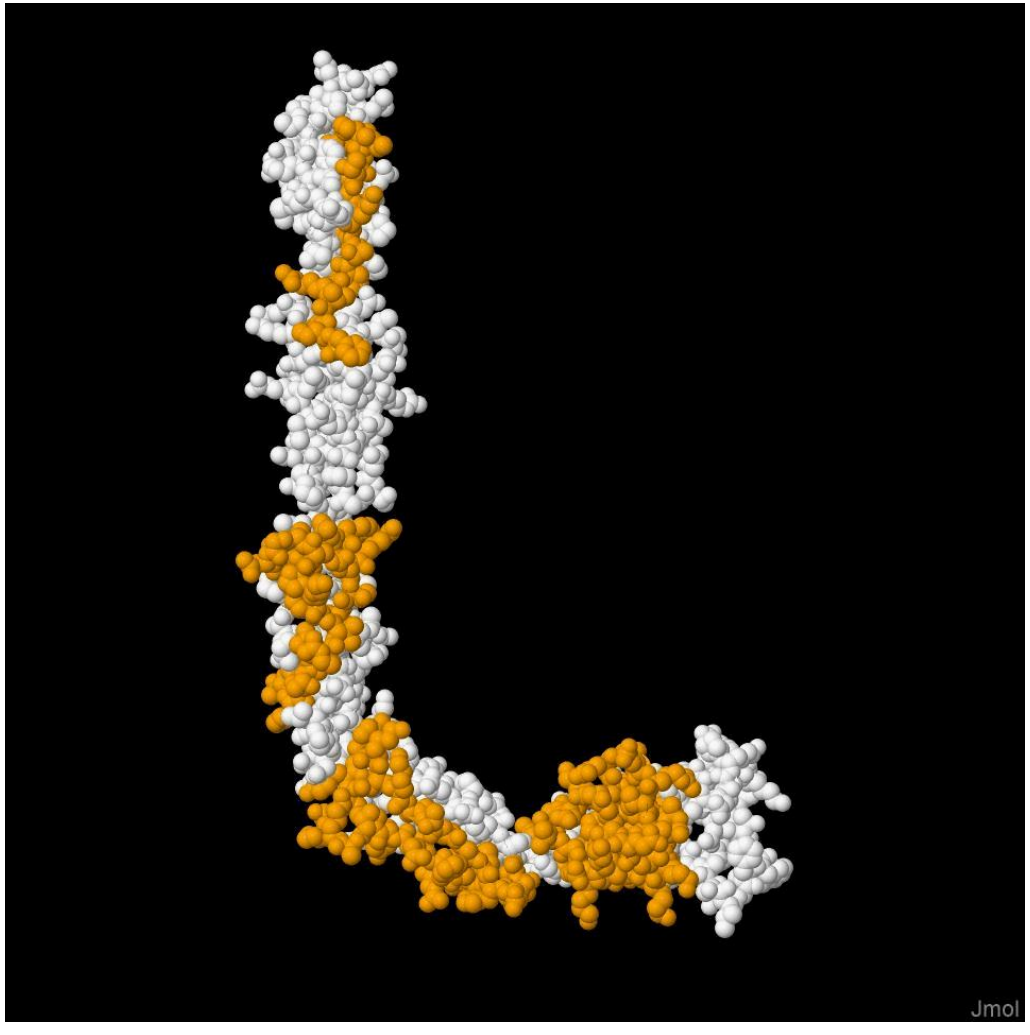
Zones recognized by antibodies represented on 3D model

L-shape

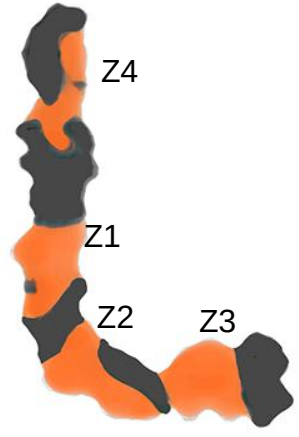


J-shape


Jmol

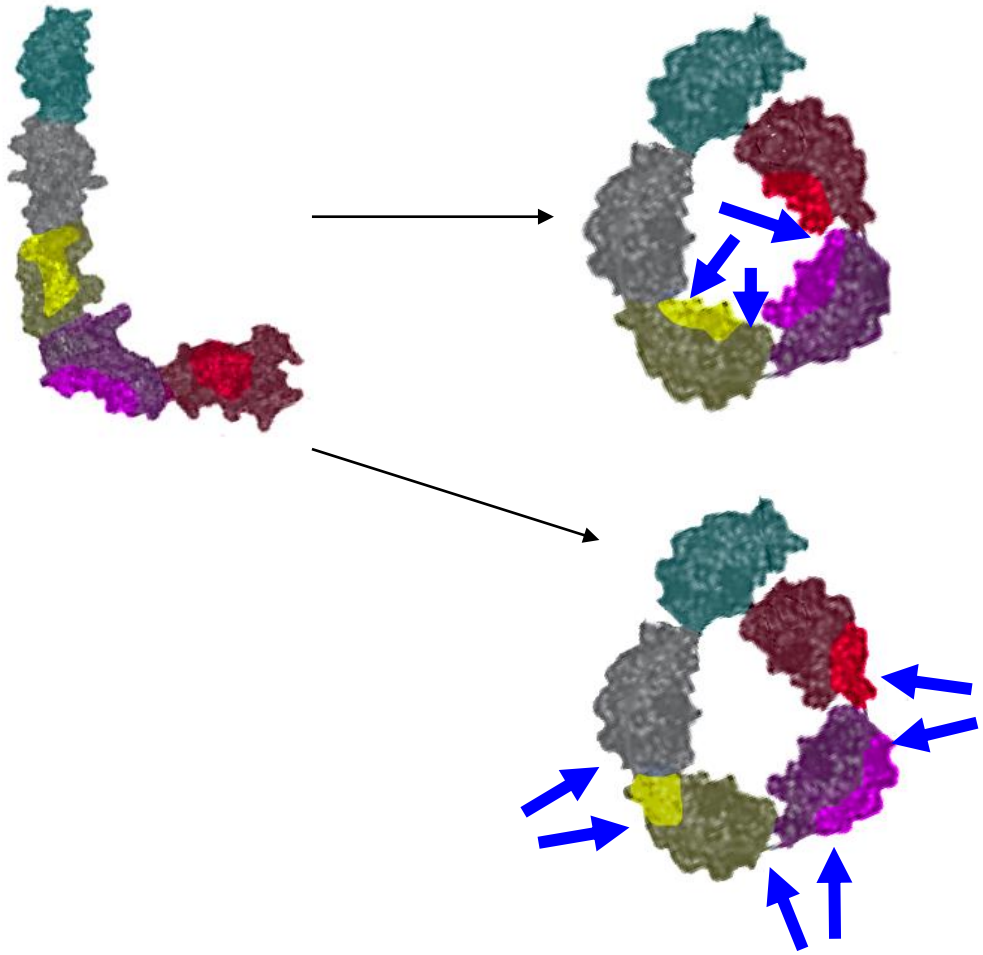


The four zones



All the peptides of each zone are represented

Change from "fish-hook" to circular form and loss of antigenicity



Model 1

After the change of conformation, the peptides remain inside the molecule and become cryptics.

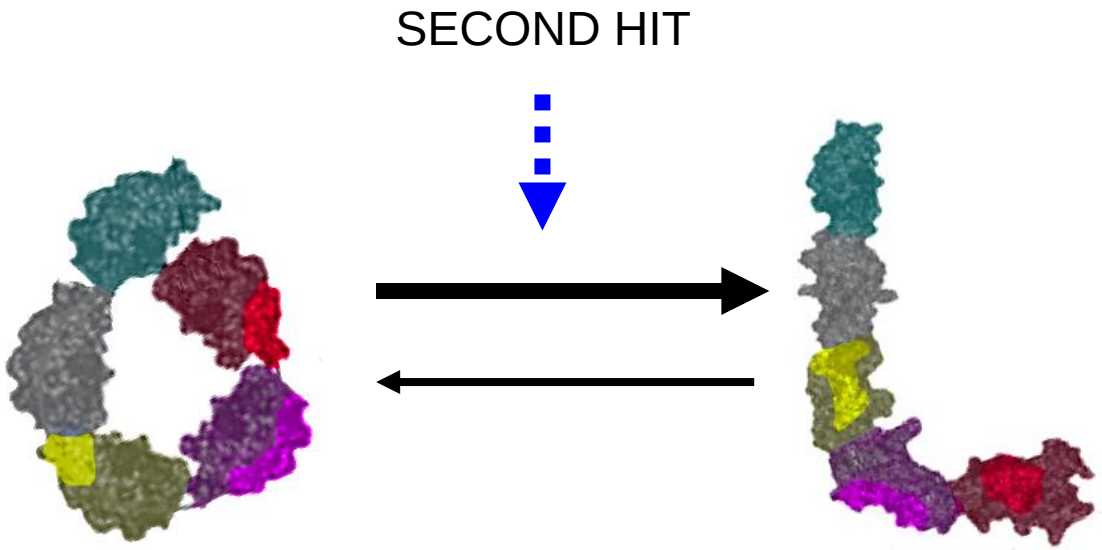
Model 2.

After the remodeling, the epitopes remain in the outer circumference of the ring, losing their antigenicity due to the deformation (stretching) of this area.

The blue arrows mark the areas that that would be subject to greater deformation by tension.

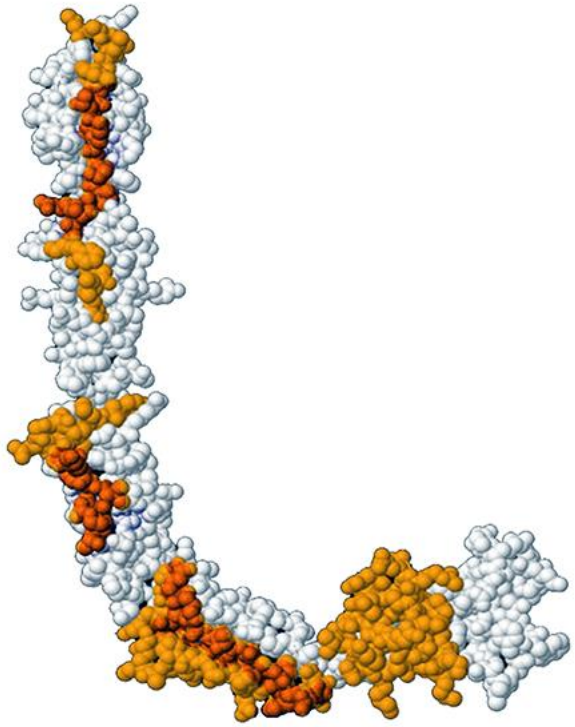
Graphic modified from: P. G. de Groot and J. C. M. Meijers. 2011 J Thromb Haemost. 9: 1275–1284

Second HIT Hipothesis an criptic epitopes shape dependent hipothesis

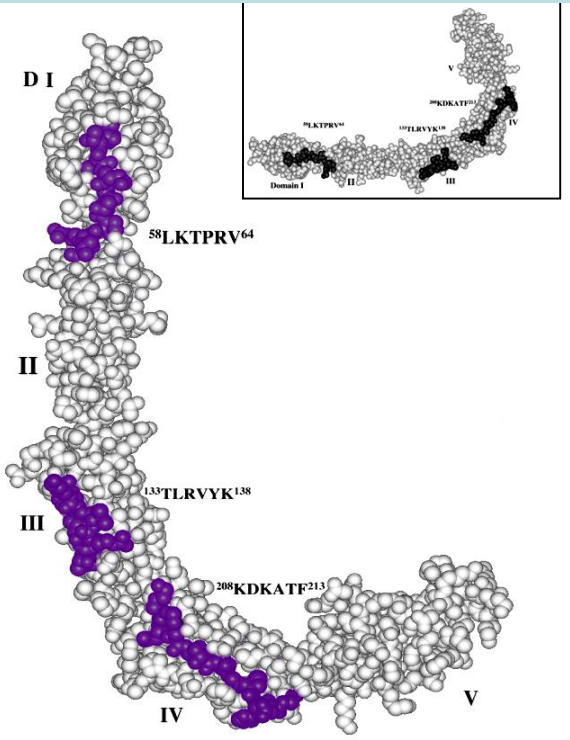


Graphic modified from: P. G. de Groot and J. C. M. Meijers. 2011 J Thromb Haemost. 9: 1275–1284

Peptides described by Serrano et al.



Hexapeptides Described by Blank et al.



Correlation of both works

-Zone 1 **P34** (peptides 33-35) Domain 3 → H3
-zone 2 **P48** (peptides 46-52) Domain 4 → ILA 3
-zone 3 **P64** (peptides 62-67). Domain 5
-Zone 4

* Modified from Blank et al. J. Clin. Immunol. 24: 12-23(2004)
The original picture can be seen in the box

Conclusions

- IgA aB2GP1 antibodies are recognize mainly 4 peptide zones

- These zones antigenic are exposed in J-shape of the protein

- Previously, these 4 zones were described as pathogenic in a in vivo model

- IgA antibodies can be considered pathogenic per se, so it must be considered as a consensus antibody

Acknowlegments

Cinderella becomes a princess, and they all lived happily ever after

Immunology Department

Margarita Sevilla
Carmen Polo
Dolores Pérez
Oscar Cabrera
Laura Naranjo
María José Castro
Sergio Mora
Daniel Pleguezuelo
Estela Paz
Antonio Serrano

Instituto de Investigacion

José Ángel Martínez
José Maria Morales
Manuel Serrano
David Lora
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