Prediction of the interacting surfaces in a trimolecular complex formed between the major dust mite allergen Der p 1, a mouse monoclonal anti-Der p 1 antibody, and its anti-idiotypic

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Abstract

Background—Two mouse monoclonal antibodies (mAbs) have been described recently; namely, mAb 2C7 (IgG2bκ), which is directed against the major house dust mite allergen Der p 1, and mAb 2G10 (IgG1κ), which is an anti-idiotypic antibody raised against mAb 2C7. The anti-idiotypic mAb 2G10 does not block the binding of mAb 2C7 to Der p 1, which means that mAb 2C7 can simultaneously bind to Der p 1 and to mAb 2G10, thereby generating a trimolecular complex consisting of antigen–idiotypic–anti-idiotypic.

Aims—To sequence and model the V region of the anti-idiotypic antibody mAb 2G10 to enable the prediction of the interacting surfaces in the trimolecular complex consisting of Der p 1–mAb 2C7–mAb 2G10.

Methods—DNA sequencing of mAb 2G10 was carried out and the Swiss Model and Swiss PDB-Viewer programs were used to build a three dimensional model of the trimolecular complex.

Results—Complementarity of shape and charge was revealed when comparing the protrusion of the previously determined Der p 1 epitope (Leu147–Gln160) with the cavity formed by the complementarity determining regions (CDRs) of mAb 2C7. Such complementarity was also observed between the mAb 2C7 epitope predicted to be recognised by mAb 2G10 (residues Lys19 from framework region 1 (FRW1) and Ser74–Gln81 from FRW3) and residues from the CDRs of mAb 2G10 (a negatively charged patch flanked by the residues Asp55H/Glu58H and Glu27L/Glu27cL). As expected, the location of the mAb 2C7 epitope recognised by mAb 2G10 does not appear to interfere with the binding of Der p 1 to mAb 2C7.

Conclusion—Although the results obtained represent only an approximation, they nevertheless provide a rare insight into how an antigen (Der p 1) might bind to its antibody (mAb 2C7) while in complex with an anti-idiotypic (mAb 2G10).

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We have recently described two mouse monoclonal antibodies; namely, mAb 2C7 (IgG2bκ),¹ which is directed against the major house dust mite allergen Der p 1, and mAb 2G10 (IgG1κ),² which is an anti-idiotypic antibody raised against mAb 2C7. We have established, using phage peptide libraries, that mAb 2C7 recognises a conformational epitope comprising Leu147–Gln160 within the Der p 1 sequence,³ and that this specificity is a major component of the human IgE anti-Der p 1 response.¹ We have also provided evidence to show that part of the epitope recognised by mAb 2G10 is a hexapeptide spanning Ser74–Tyr79 within the framework 3 (FRW3) region of mAb 2C7.²

Anti-idiotypic mAb 2G10 appears to be directed against FRW sequences encoded by the V_H3 and V_H4 gene families, but its most intriguing property is that it reacts with human IgE regardless of its antigenic specificity.² The fact that mAb 2G10 binds to the humanised (complementarity determining region (CDR) grafted) CAMPATH-1H antibody,⁴ but not to the original rat CAMPATH-1 YTH34.5.6 antibody,⁴ is further evidence that it is directed against a FRW region, rather than the CDRs.² This is of course in keeping with our observation that mAb 2G10 does not block the binding of mAb 2C7 or human IgE to Der p 1.² This means that mAb 2C7 can simultaneously bind to Der p 1 and to mAb 2G10, thereby generating a trimolecular complex consisting of antigen–idiotypic–anti-idiotypic. This, therefore, raises the question of the relative disposition of these three molecules in this immune complex, which can only be answered with confidence by solving its crystal structure. There have been only four reports describing co-crystals of idiotypic–anti-idiotypic complexes in the literature.^{5–8} Two of these are based on the anti-idiotypic mimicking the antigen and therefore reacting with the CDRs rather than the FRW regions,^{5,6} and the other two anti-idiotypes recognise CDR and FRW residues.^{7,8} As mentioned above, mAb 2G10 recognises only FRW residues, and as such it has a rather special specificity.

Powerful computer packages for three dimensional modelling and molecular docking are currently available. Using the programs Swiss Model^{9,10} and Swiss PDB-Viewer,^{9,10} we have investigated shape and charge complementarity to predict the interacting surfaces in the trimolecular complex

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Figure 1 A 1% agarose gel showing PCR products and restriction digests of plasmids containing the monoclonal antibody 2G10 cDNA inserts for the light chain (top lanes 2–9) and the heavy chain Fd fragment (bottom lanes 2–9). Lane M contains a 100 bp ladder.

consisting of Der p 1–mAb 2C7–mAb 2G10. Although these results are only an approximation, our molecular modelling efforts, combined with our previous experimental data,^{1–3} provide a rare insight into how an antigen might bind to its antibody while in complex with an anti-idiotypic.

Materials and methods

PRODUCTION OF MOUSE MONOCLONAL ANTI-IDIOTYPE 2G10

The anti-idiotypic mAb 2G10 (IgG1 κ) was produced and characterised as documented previously.²

REVERSE TRANSCRIPTION AND POLYMERASE CHAIN REACTION (PCR)

Approximately 1×10^6 mAb 2G10 hybridoma cells were harvested by centrifugation and

homogenised using a QIASHREDDERTM column (Qiagen Limited, Dorking, UK). Total RNA was prepared using the Qiagen RNeasy kit according to the manufacturer's instructions. cDNA synthesis was performed by heating 10 μ l of the total RNA preparation to 70°C for five minutes, then placing it on ice and adding 4 μ l 5 $\times$ Superscript buffer, 0.5 μ l oligo(dT) primer (0.5 mg/ml; Pharmacia, Uppsala, Sweden), 2 μ l dNTP (5 mM), 2 μ l dithiothreitol (0.1 M), 0.6 μ l RNasinTM ribonuclease inhibitor (40 U/ml; Promega Corporation, Madison, Wisconsin, USA), and 200 U Superscript IITM Rnase H⁻ reverse transcriptase (Gibco BRL, Middlesex, UK). This was followed by incubation at 42°C for one hour.

PCR was performed as described,¹¹ using 1 μ l of cDNA in each reaction and the primers documented elsewhere¹¹ to amplify the complete κ chain cDNA and the heavy chain Fd fragment cDNA. PCR products were run on a 1% agarose gel containing ethidium bromide (0.2 μ g/ml) and the DNA was visualised using an ultraviolet transilluminator. Molecular weight markers were a 100 bp DNA ladder (Promega Corporation).

CLONING PCR PRODUCTS

Ligation was performed using a TA cloning kit (Invitrogen, San Diego, California, USA) with the pCRTM 2.1 plasmid. Transformation of INVaF⁺ One ShotTM competent cells was carried out by heat shock. All procedures were carried out according to the manufacturer's instructions. Transformed cells were selected on the basis of blue/white colour selection in the presence of X-gal. Plasmid DNA was purified from 3 ml overnight cultures in Luria-Bertani (LB) medium containing ampicillin and methicillin (20 and 80 μ g/ml, respectively), using QIA prep spin miniprep kit (Qiagen Limited). Purified plasmid was digested with EcoRI to check for the presence of an insert of the correct size. The restriction digest was analysed on a 1% agarose gel.

DNA SEQUENCING

Automated DNA sequencing was carried out on an ABI PRISMTM 310 genetic analyser, using the ABI PRISM dye terminator cycle sequencing ready reaction kit (Perkin-Elmer Applied Biosystems, Warrington, UK).

	10	20	27abcd	30	40	50	60	70	80	90	100
2G10	NIVLTQFPTSLAVSPGQRATISCRASESVEYFGTGLMQWYQKPKGQPKLLIYTASNVEGVPARFSGSGGTDNFIHPVEEDDIAMFYCQSRKVPSTFGGGTRELKIR										
1MF2	DT---S-A-----L-----D-Y-KSF-N-F-----A---QG-----S-H---M---S-----KE---W-----K---										
1ACY	D--M--S-A--V--L-----DSY-KSF-H-----V---I---L-----R--T-T-D---A--A-T-Y--NNED-P---A--K---M---										
1GGB	D---S-G-----L-----DDD-NSFLH-----RS--LI--I-D-----R--T-T-N---A--V-T-Y--NED-L---A--K---										

	10	20	30	40	52a	60	70	82abc	90	100abc	110
2G10	DVQLVQSGPELVKPGAAMKSCRASGYTFTSHVMHWKQKPGQGLEWIGYINPYNDYSENNEKFKDKATLSDTSSTAYMELNSLTSEDSAVYYCAKAGYNN...YYAMEYWGQGTSTVTS										
1AE6	QI--Q-----S--I--K-----DYYIN-M-----W-D-GSGNTKY-----G-----V-----Q-S-----T--F--REKTTY...D-----										
1PLG	QI--Q-----R--S--I--K-----DYYI-----R--E-----W-Y-GSGNTKY-----G-----V-----Q-S-----F--RG-K...F--D-----										
1FBI	Q--Q-P-A-----S--L--K-----YW-----G-----E-D-SDS-PNY-----G-----V-K-----Q-S-----SLY-YGTS-GVLD-----										

Figure 2 Sequence alignments of the monoclonal antibody 2G10 V_L (top panel) and V_H (bottom panel) regions with homologous V_L (1MF2,³⁰ 1ACY,³³ and 1GGB³⁴) and V_H (1AE6,³¹ 1PLG,³⁵ and 1FBI³⁶) sequences having structural coordinates available within the protein databank.¹⁸ Complementarity determining regions (CDRs) are indicated by solid lines above the sequences; dots indicate missing amino acids.

Table 1 Canonical structure classification of the hypervariable loop regions of monoclonal antibody 2G10² V_L and V_H according to Clothia and co-workers,³⁷ showing sequence similarities with known antibody structures (McP603,⁴² HyHeL45,⁴⁰ and NC41⁴¹)

Canonical structure		CDR residues														FRW residues			
		25	26	27	a	b	c	d	e	f	28	29	30	31	32	33			
L1 region		25*	26	27	28	29*	30	31	a	b	c	d	e	f	32	33*		2	71
2G10		A	S	E	S	V	E	Y	–	–	F	G	T	G	L	M		2*	71*
McP603	3	S	S	E	S	L	L	N	S	G	N	E	K	N	F	L		I	F
L2 region		50	51	52														48*	64*
2G10		T	A	S														I	G
McP603	1	G	A	S														I	G
L3 region		90*	91	92	93	94	95*	96*											
2G10		Q	S	R	K	V	P	S											
McP603	1	N	D	H	S	Y	P	L											
H1 region		31	32	34*													26*	27*	28
2G10		S	H	M													G	Y	T
McP603	1	S	Y	M													G	F	I
H2 region		52a	b	c	53	54	55*												
2G10		P	–	–	Y	N	D												71*
HyHeL5	2	P	–	–	G	S	G												S
NC41		T	–	–	N	T	G												A
																			L

Apart from the L1 region, the Kabat¹³ and Clothia³⁷ systems agree in terms of amino acid numbering of the hypervariable loop regions.

Numbers in bold represent residue positions according to the Kabat system.¹³

*Canonical determinants.

FRW, framework region.

SEQUENCE ANALYSIS OF mAb 2G10

The mAb 2G10 heavy and light chain V region sequences were compared with murine nucleotide sequences using DNAPLOT, which is available at the International ImmunoGeneTics database (<http://imgt.cines.fr:8104>).¹² Sequence numbering was done according to the Kabat system.¹³

SEQUENCE ANALYSIS OF ANTIBODIES TESTED FOR REACTIVITY WITH mAb 2G10

Comparison of the mAb 2C7 V region sequence with those of antibodies tested for reactivity with mAb 2G10² was carried out using Gene Doc (Nicholas KB, Nicholas HB Jr, 1997, Gene Doc: analysis and visualisation of genetic variation, <http://www.cris.com/~ketchup/genedoc.shtml>).

MOLECULAR MODELLING

We have previously described molecular modelling of mAb 2C7.¹ Molecular models of mAbs CAMPATH-1H⁴ (1BEY),¹⁴ CAMPATH-1H YTH34.5.6⁴ (1BFO),¹⁴ and MOPC-21^{15 16} (1IGC)¹⁷ were available from the protein databank (PDB).¹⁸ Modelling of mAbs 105AD7,¹⁹ A2,^{20 21} Cal-4G^{22 23} and MOPC-141^{24 25} were based on the following

templates that are available from the PDB¹⁸: 1FVD,²⁶ 1HOU,²⁷ 7FAB,²⁸ and 1OAK.²⁹ Modelling of mAb 2G10² V_L and V_H was based on 1MF2³⁰ and 1AE6,³¹ respectively. All modellings were carried out using Swiss Model^{9 10} and were viewed using Swiss PDB-Viewer.^{9 10} Molecular modelling of Der p 1 has been described previously,³² and the Der p 1 epitope (Leu147–Gln160) recognised by mAb 2C7 has been reported by us elsewhere.³

Results

PCR PRODUCTS AND CLONING

PCR amplification of mAb 2G10 cDNA, using the light and heavy chain primers, in conjunction with the reverse κ and γ chain primers, resulted in a single band of approximately 650 bp with V_Lfr1b and V_Hfr1g, respectively. These products were cloned into the vector pCRTM 2.1 and 10 clones from each reaction were selected for restriction analysis of the plasmids. Figure 1 shows the results of this analysis for eight representative clones for the heavy chain and eight for the κ chain.

SEQUENCE ANALYSIS OF mAb 2G10

Figure 2 shows the cDNA deduced protein sequences of the light and heavy chains of mAb 2G10. These are the sequences of individual clones that were identified as containing no PCR errors by comparison with other clones and with the Kabat database.¹³ The first eight amino acids of the light chain and the first nine of the heavy chain were determined by the degenerate primers used for PCR.¹¹ The mAb 2G10 heavy and light chain V region sequences showed the greatest homology with members of the IGHV1 and IGKV3 murine gene families, respectively. According to the Kabat system,¹³ mAb 2G10 V_L has an insertion of four residues, 27a to 27d, in the first hypervariable region (L-CDR1). Insertions of one residue, 52a, and three residues, 100a to 100c, occur in the second (H-CDR2) and third (H-CDR3)

Table 2 Sequence analysis of the FRW1 and FRW3 regions of monoclonal antibodies (mAbs) tested for reactivity with mAb 2G10

Antibody	Heavy chain										Reactivity with mAb 2G10	Refs
	FRW1	FRW3										
2C7	19	74							81			
Cal-4G	K	S	K	N	T	L	Y	L	Q	+	1	
A2	S	S	K	N	Q	F	S	L	K	+	22, 23	
CAMPATH-1H	R	S	K	N	T	L	Y	L	Q	+	20, 21	
105AD7	S	S	K	N	Q	F	S	L	R	+	4	
MOPC-21	T	S	K	N	Q	V	V	L	T	—	19	
MOPC-141	K	P	K	N	T	L	F	L	Q	—	15, 16	
YTH34.5.6	S	S	K	S	Q	V	F	L	K	—	24, 25	
	R	T	Q	N	M	L	Y	L	Q	—	4	

Differences in shape and polarity of residues at positions 19 and 74–81 may hinder reactivity with mAb 2G10.

Polar amino acids are shown in bold.

FRW, framework region.

Figure 3 (A) The predicted epitope on monoclonal antibody (mAb) 2G10, shown in backbone representation (polar residues are shown in cyano blue and non-polar residues in red). (B) Side view of the Fv region of mAb 2C7 showing the location of the predicted epitope on framework region 3 (FRW3) of V_H (dark blue). (C) Differences in shape and polarity (shown in space filling representation) between mAb 2G10 reactive (upper row) and non-reactive (lower row) antibodies. Amino acids that are different from those of mAb 2C7 are highlighted

hypervariable regions of the mAb 2G10 V_H, respectively. Analysis of the FRW regions showed the presence of an insertion of three residues, 82a to 82c, in the FRW3 region of mAb 2G10 V_H. No insertions or deletions were found in the other FRW regions or in L-CDR2, L-CDR3, and H-CDR1 (fig 2).

CANONICAL STRUCTURE ANALYSIS OF THE mAb 2G10 HYPERVARIABLE LOOP REGIONS

Analysis of the hypervariable loop regions was performed according to the canonical structure hypothesis of Clothia and co-workers.³⁷ The L1 region is formed by residues Ala25 to Met33 from L-CDR1 and FRW residues Ile2 and Phe71, corresponding to the canonical structure 3.³⁷ The L2 region involves residues Thr50, Ala51, and Ser52 from L-CDR2 and FRW residues Ile48 and Gly64, and adopts the unique conformation seen among all other structures reported previously.³⁸ The L3 region involves residues Gln90, Ser91, Arg92, Lys93, Val94, Pro95, and Ser96, and is most closely related to the canonical structure 1. The H1 region involves H-CDR1 residues Ser31, His32, and Met34 and FRW residues Gly26, Tyr27, Thr28, Phe29, Thr30, and Lys94, and is similar to the canonical structure 1. The H2 region is defined by residues at positions 52a/53 to 55, and a major determinant of its

conformation is the FRW residue at position 71.³⁹ Thus, the mAb 2G10 H2 region (Pro52a, Tyr53, Asn54, Asp55, and Ser71) can be compared with mAbs HyHel45⁴⁰ and NC41,⁴¹ corresponding to the canonical structure 2, which allows the presence of a small or medium size residue at position 71.³⁹ The L1, L2, L3, and H1 regions of mAb 2G10 have the same canonical structure as mouse mAb McP603.⁴² Despite recent progress,⁴³ the conformation of the H3 region has not yet been fully classified, therefore leading to major uncertainties concerning the orientation of the mAb 2G10 H-CDR3. Table 1 shows the canonical structure classification of the mAb 2G10 hypervariable loop regions.

A MOLECULAR MODEL OF THE VARIABLE REGION OF mAb 2G10

A predicted three dimensional structure of the mAb 2G10 Fv (fragment variable) region was constructed by modelling on homologous structures within the PDB.¹⁸ A search of the PDB database was performed using the BLAST network service at NCBI, and this produced several structures with reasonable homology to the mAb 2G10 light chain (1MF2,³⁰ 1ACY,³³ and 1GGB³⁴) and heavy chain (1AE6,³¹ 1PLG,³⁵ and 1FBI³⁶) (fig 2). 1MF2 (F11.2.32),³⁰ 1ACY (59.1),³³ and

Figure 4 (A) Front and (B) side views of the Der p 1-mAb 2C7 complex showing shape complementarity between the Der p 1 (top structure) epitope recognised by monoclonal antibody (mAb) 2C7 (Leu147-Gln160)³ and the Fv region of mAb 2C7 (lower structure). The contacting surfaces between (C) Der p 1 and (D) mAb 2C7 are defined by a quadrangular patch (blue), which in Der p 1 is surrounded by residues Arg151, Arg156, Gln118, and Gln166 and in mAb 2C7 by residues Pro61H, Thr5L, Trp103H, and Lys 75H. The previously determined epitope for mAb 2C7 on Der p 1 is shown in purple.

1GGB (50.1)³⁴ are all murine monoclonal antihuman immunodeficiency virus antibodies. Sequence analysis of L-CDR1 showed that mAb 2G10 has greater homology with F11.2.32 and 59.1 than with 50.1. Although L-CDR1 of F11.2.32 has only one amino acid difference when compared with 59.1 (TyrL27d → Ser), a Tyr at this position was shown to cause structural differences in L-CDR1 affecting antigen recognition.³⁰ Because mAb 2G10 also has TyrL27d, modelling of its light chain was based on F11.2.32 V_L, which also belongs to the murine VK3 subgroup and corresponds to the same canonical classification as mAb 2G10 L regions³⁰ (fig 2). Another template was chosen for modelling the V_H region of mAb 2G10, because F11.2.32 does not have the same length and canonical structure classification as the mAb 2G10 H2 and H3 loop regions.³⁰ Thus, modelling of the mAb 2G10 V_H region was based on 1AE6 (antitumour mAb CTM01),³¹ which shows sequence similarities and has the same loop length (H3 region inclusive) compared with the other homologous structures (1PLG³⁵ and 1FBI³⁶) (fig 2). The models were built using Swiss Model^{9 10} and viewed using Swiss PDB-Viewer^{9 10}

ANALYSIS OF THE MOLECULAR MODELS OF ANTIBODIES THAT WERE TESTED FOR REACTIVITY WITH mAb 2G10

Competitive inhibition studies previously confirmed that mAb 2G10 is most likely to be directed against FRW regions encoded by the V_H3 and V_H4 genes.² It is known that codons

6–24 in FRW1 and 67–85 in FRW3 are highly conserved among sequences within a single V_H family, but diverge when compared with different V_H families.⁴⁴ The variation of these sequences in FRW1 and FRW3 is considerably smaller when comparing mouse and human V_H3 gene segments.⁴⁴ Considering that mAb 2C7 is a member of the X-24 mouse family, and this family shows up to 70% homology with the human V_H3 family,¹ we speculate that sequence variation affecting polarity and structure within FRW1 and FRW3 might explain differential reactivity with mAb 2G10 (table 2). We have already reported the sequence spanning amino acids 74–79 within FRW3 as being part of the epitope recognised by mAb 2G10.² Comparison of the three dimensional models of the mAb 2G10 reactive and non-reactive antibodies, using Swiss Model^{9 10} and Swiss PDB-Viewer,^{9 10} showed differences in structure and polarity of the side chains of amino acid residues spanning positions 74–81 within FRW3 and at position 19 in FRW1. It seems that the mAb 2C7 epitope recognised by mAb 2G10 is a protrusion consisting of polar amino acids Ser74-Gln81 and Lys19 (fig 3). In mAbs Cal-4G^{22 23} and CAMPATH-1H,⁴ which were reactive with mAb 2G10, the lack of a positively charged amino acid (Lys/Arg → Ser) in position 19 seems to be structurally compensated for by the presence of a positively charged amino acid at position 81 (Gln → Lys/Arg). Monoclonal antibodies 105AD7,¹⁹ MOPC-21,^{15 16} and MOPC-141,^{24 25} which were not reactive with mAb 2G10,² all have non-polar, rather than polar, residues in

position 79 (Val or Phe), which might lead to loss of reactivity. Monoclonal antibody 105AD7¹⁹ has a polar amino acid (Thr) at positions 19 and 81, which might hinder reactivity with mAb 2G10. Moreover, mAb MOPC-21^{12,13} has a non-polar residue in position 74, namely Pro, that might also influence its reactivity with mAb 2G10,² because Ser in this position appears to be highly conserved in mAb 2G10 reactive antibodies from the V_H3 and V_H4 gene families. In the original rat CAMPATH-1H YTH34.5.6 antibody,⁴ the presence of Gln, instead of Lys, in position 75 and of a non-polar residue in position 77 (Met), instead of a polar residue (Thr/Gln), might explain the lack of reactivity with mAb 2G10 (table 2; fig 3).

ANALYSIS OF THE MOLECULAR INTERACTIONS WITHIN THE TRIMOLECULAR COMPLEX Der p 1-mAb 2C7-mAb 2G10

The Der p 1 epitope recognised by mAb 2C7 was previously determined using phage display libraries and was shown to be a conformational sequence comprising Leu147-Gln160.³ Possible interacting surfaces between mAb 2C7 and Der p 1 were predicted by analysing the complementarity in charge and structure between the previously determined Der p 1 epitope Leu147-Gln160³ and mAb 2C7 CDRs using Swiss PDB-Viewer.^{9,10} Analysis of the molecular model of the complex formed

between Der p 1³ and mAb 2C7¹ revealed a high degree of surface complementarity (fig 4). The Der p 1 surface contacting mAb 2C7 consists of four residues forming a quadrangle; namely, Arg151, Arg156, Gln118, and Gln166. The complementary quadrangle on mAb 2C7 is formed by residues Pro61H, Thr5L, Trp103H, and Lys75H. The previously determined Der p 1 epitope (Leu147-Gln160)³ forms the most protruding part of the Der p 1 surface involved in the interaction with mAb 2C7. Such protrusion from the Der p 1 surface appears to fit into a cavity formed by the CDRs of mAb 2C7 and defined by the solvent accessible triad Lys64H, Arg24L, and Arg97H. The interacting surfaces between mAb 2C7 and Der p 1 also revealed complementary electrostatic potential (fig 5). The mAb 2C7 contacting surface on Der p 1 is formed by a patch of negatively charged residues surrounded by four positively charged residues; namely, Arg151, Arg156, Arg161, and Lys145. This negatively charged patch is met on the mAb 2C7 surface by a positively charged area surrounded by the negatively charged residues Asp53H, Asp53L, and Asp28H. The positively charged Der p 1 residues Arg156 and Arg161 are complementary to the negatively charged mAb2C7 residues Asp53H and Asp28H, respectively. The protruding Der p 1 residues Lys145 and Arg151 fit into two cavities formed between the

Figure 5 Complementarity of (A) shape and (B and C) electrostatic potential between the monoclonal antibody (mAb) 2C7 epitope on Der p 1 and the Fv region of mAb 2C7. The protrusion formed by the Der p 1 epitope (Leu147-Gln160)³ fits into the cavity defined by the solvent accessible residues (Lys64H, Arg24L, and Arg97H) of the complementarity determining regions (CDRs) of mAb 2C7. Complementarity of electrostatic potential between mAb 2C7 (a negatively charged patch defined by residues Asp53H, Asp53L, and Asp28H) (B) and Der p 1 (a positively charged patch defined by residues Arg151, Arg156, Arg161, and Lys145) (C) is shown in red and blue, respectively. The mAb 2C7 residues Asp53H and Asp28H (B) are complementary to the Der p 1 residues Arg156 and Arg161 (C), respectively. The protruding Der p 1 residues Lys145 and Arg151 fit into two cavities formed between the mAb 2C7 residues Asp28H and Asp53H on one side and Asp28H, Asp53H, and Asp53L on the other side. The Fv region of mAb 2C7 (A) is coloured according to solvent accessibility, decreasing in the order yellow, green, light blue, and dark blue. The electrostatic potential was computed by the program Swiss PDB-Viewer,^{9,10} which uses simple coulomb interaction.

Figure 6 A molecular model of the trimolecular complex consisting of Der p 1–monoclonal antibody (mAb) 2C7–mAb 2G10. The previously determined Der p 1 epitope recognised by mAb 2C7 (Leu147–Gln160),³ shown in space filling representation (purple). The mAb 2C7 epitope predicted to be recognised by mAb 2G10 forms a protrusion, involving residues Ser74–Gln81 (framework region 3 (FRW3)) and Lys19 (FRW1), which fits into a cavity, defined by residues Asp55H and Glu58H, on the complementarity determining regions (CDRs) of mAb 2G10. (A) Side and (B) front views of the predicted mAb 2G10 epitope on mAb 2C7 and the CDRs of mAb 2G10, shown in space filling representation and coloured according to solvent accessibility, decreasing in the order yellow, green, light blue, and dark blue. (C) Side and (D) front views of the trimolecular complex represented by its molecular surfaces and coloured according to electrostatic potential. Again, the electrostatic potential was computed by the program Swiss PDB-Viewer,^{9, 10} which uses simple coulomb interaction. The mAb 2C7 epitope predicted to be recognised by mAb 2G10 consists of a positively charged protrusion (blue), which fits into a negatively charged cavity (red) on the CDRs of mAb 2G10. The positively charged mAb 2C7 residues Lys75H and Lys19H seem to fit into two cavities formed between the mAb 2G10 negatively charged residues (coloured pink) Asp55H/Glu58H and Glu27L/Glu27cL, respectively. In (A) and (C) mAb 2G10 is pulled apart from the Der p 1–mAb 2C7 complex and viewed from the side. In (B) and (D) each component is rotated 90° towards the viewer.

mAb 2C7 residues Asp28H and Asp53H on one side and Asp28H/Asp53H and Asp53L on the other side.

Figure 6 shows the predicted interaction surfaces within the trimolecular complex of Der p 1–mAb 2C7–mAb 2G10. Shape and charge complementarity was observed between the predicted epitope on mAb 2C7 and the CDRs of mAb 2G10. The mAb 2C7 epitope recognised by mAb 2G10 is formed by a positively charged protrusion defined by heavy chain residues within the FRW3 region; namely, Ser74–Gln81 and Lys19 from FRW1, which are complementary to a negatively charged surface on mAb 2G10 CDRs. The positively charged mAb 2C7 residues Lys75H and Lys19H seem to fit into two cavities formed between the mAb 2G10 negatively charged residues Asp55H/Glu58H and Glu27L/Glu27cL, respectively (fig 6). Furthermore, the epitope recognised by mAb 2G10 is located on the FRW region of mAb 2C7 and is away from the CDR residues that are thought to form the groove responsible for binding to Der p 1. As expected, the location of the mAb 2C7 epitope recognised by mAb 2G10 does

not appear to interfere with the binding of Der p 1 to mAb 2C7, which supports our previous experimental data showing that mAb 2G10 does not block the binding of Der p 1 to mAb 2C7.²

Discussion

In this paper, we used the programs Swiss Model^{9, 10} and Swiss PDB-Viewer^{9, 10} to build a three dimensional model of a trimolecular complex consisting of Der p 1–mAb 2C7–mAb 2G10. This task was facilitated by the availability of previously published experimental data, which defined the mAb 2C7 epitope on Der p 1 (Leu147–Gln160),³ and which demonstrated that mAb 2G10 does not bind to the CDR regions of mAb 2C7.² This helped us to focus our efforts on the FRW regions of mAb 2C7 as the site of interaction with mAb 2G10. Investigation of shape and charge complementarity suggested that the mAb 2C7 epitope recognised by mAb 2G10 is distant from the mAb 2C7 CDR regions that are involved in Der p 1 binding. This means that Der p 1 and mAb 2G10 could engage mAb 2C7 simultaneously, which is in keeping

with our previous experimental data showing that mAb 2G10 does not block the binding of Der p 1 to mAb 2C7.²

Given that the anti-idiotypic mAb 2G10 recognises FRW residues encoding human immunoglobulin V_H3 and V_H4 gene segments,² it might be feasible to use this anti-idiotypic in inhibition experiments to determine the influence of FRW regions of such antibodies on antigen binding. Furthermore, considering its broad IgE specificity,² our anti-idiotypic mAb 2G10 could potentially have immunomodulatory applications. For instance, a chimaeric human IgG version of mAb 2G10 might prove to be a useful molecule for binding to mast cell FcεRI bound IgE, and in doing so co-ligating FcεRI with FcγRIIb, which has been reported to be a negative regulator of type I allergic responses.^{45,46} Thus, having cloned and sequenced the variable region of mAb 2G10, it will now be relatively straightforward to produce a chimaeric human IgG construct of it. This will provide new opportunities for investigating the potential ability of this anti-idiotypic to modify IgE mediated allergic responses.

Clearly, the results obtained here represent only an approximation, but nevertheless they provide a rare insight into how a clinically important allergen (Der p 1) might bind to an antibody representative of human IgE (mAb 2C7) while complexed with an anti-idiotypic (mAb 2G10).

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- McElveen JE, Clark MR, Smith SJ, *et al.* Primary sequence and molecular model of the variable region of a mouse monoclonal anti-Der p 1 antibody showing a similar epitope specificity as human IgE. *Clin Exp Allergy* 1998;28:1427–34.
- McElveen JE, Furtado PB, Smith SJ, *et al.* Characterisation of a mouse monoclonal anti-idiotypic reactive with a V-region sequence commonly used by human immunoglobulins. *J Clin Pathol: Mol Pathol* 2000;53:77–82.
- Furmonaviciene R, Tighe PJ, Clark MR, *et al.* The use of phage-peptide libraries to define the epitope specificity of a mouse monoclonal anti-Der p 1 antibody representative of a major component of the human immunoglobulin E anti-Der p 1 response. *Clin Exp Allergy* 1999;29:1563–71.
- Riechmann L, Clark MR, Waldmann H, *et al.* Reshaping human antibodies for therapy. *Nature* 1988;332:323–7.
- Evans SV, Rose DR, To R, *et al.* Exploring the mimicry of polysaccharide antigens by anti-idiotypic antibodies. The crystallization, molecular replacement, and refinement to 2.8 Å resolution of an idiotope-anti-idiotypic Fab complex and of the unliganded anti-idiotypic Fab. *J Mol Biol* 1994;241:691–705.
- Fields BA, Goldbaum FA, Ysern X, *et al.* Molecular basis of antigen mimicry by an anti-idiotypic. *Nature* 1995;374:739–42.
- Bentley GA, Boulton G, Riottot MM, *et al.* Three-dimensional structure of an idiotope-anti-idiotypic complex. *Nature* 1990;348:254–7.
- Ban N, Escobar C, Garcia R, *et al.* Crystal structure of idiotope-anti-idiotypic Fab complex. *Proc Natl Acad Sci U S A* 1994;91:1604–8.
- Guex N, Peitsch MC. SWISS-MODEL and the Swiss-Pdb Viewer: an environment for comparative protein modelling. *Electrophoresis* 1997;18:2714–23.
- Peitsch MC. Pro-Mod and Swiss-Model: internet-based tools for automated comparative protein modelling. *Biochem Soc Trans* 1996;24:274–9.
- Kettleborough CA, Saldanha J, Ansell KH, *et al.* Optimization of primers for cloning libraries of mouse immunoglobulin genes using the polymerase chain reaction. *Eur J Immunol* 1993;23:206–11.
- LeFranc M-P, Giudicelli V, Ginestoux C, *et al.* IMGT, the international ImMunoGeneTics database. *Nucleic Acids Res* 1999;27:209–12.
- Kabat EA, Wu TT, Reid-Miller M, *et al.* *Sequences of proteins of immunological interest*, 5th ed. Bethesda: US Department of Health and Human Services, 1991.
- Cheetham GM, Hale G, Waldmann A, *et al.* Crystal structures of a rat antibody CD52 (CAMPATH-1H) therapeutic antibody Fab fragment and its humanised counterpart. *J Mol Biol* 1998;284:85–99.
- Bothwell ALM, Paskind M, Reth M, *et al.* Heavy chain variable region contribution to the b-NP family of antibodies: somatic mutation evident in a gamma-2a variable region. *Cell* 1981;24:625–37.
- Hamlyn PH, Gait MJ, Milstein C. Complete sequence of an immunoglobulin mRNA using specific priming and the dideoxynucleotide method of RNA sequencing. *Nucleic Acids Res* 1981;9:4485–94.
- Derrick JP, Wigley DB. The third IgG-binding domain from streptococcal protein G. An analysis by X-ray crystallography of the structure alone and in a complex with Fab. *J Mol Biol* 1994;243:906–18.
- Berman HM, Westbrook J, Feng Z, *et al.* The protein data-bank. *Nucleic Acids Res* 2000;28:235–42.
- Austin EB, Robins RA, Durrant LG, *et al.* Human monoclonal anti-idiotypic antibody to the tumour-associated antibody 791T/36. *Immunology* 1989;67:525–30.
- Potter KN, Yucheng L, Capra JD. Staphylococcal protein A simultaneously interacts with framework region 1, complementarity-determining region 2 and framework region 3 on human VH3-encoded Igs. *J Immunol* 1996;157:2982–8.
- Sanz I, Capra DJ. Vκ and Jκ gene segments of A/J Ars-A antibodies: somatic recombination generates the essential arginine at the junction of the variable and joining regions. *Proc Natl Acad Sci U S A* 1987;84:1085–9.
- Spellerberg MB, Chapman CJ, Mackridge I, *et al.* Dual recognition of lipid A and DNA by human antibodies encoded by the Vh4-21 gene: a possible link between infection and lupus. *Hum Antibodies Hybridomas* 1995;6:52–6.
- Williams SC, Winter G. Cloning and sequencing of human immunoglobulin Vλ gene segments. *Eur J Immunol* 1993;23:1456–61.
- Sakano H, Maki R, Kurosawa Y, *et al.* Two types of somatic recombination are necessary for the generation of complete immunoglobulin heavy chain genes. *Nature* 1980;286:676–83.
- Potter M, Newell JB, Rudikoff R, *et al.* Classification of mouse Vκ groups based on the partial amino acid sequence to the first invariant tryptophan: impact of 14 new sequences from IgG myeloma proteins. *Mol Immunol* 1982;19:1619–30.
- Eigenbrot C, Randal M, Presta L, *et al.* X-ray structures of the antigen-binding domains from three variants of humanised anti-P185HER2 antibody 4D5 and comparison with molecular modelling. *J Mol Biol* 1993;229:969–95.
- Hougs L, Juul L, Sveigaard A, *et al.* Structural requirements of the major protective antibody to Haemophilus influenzae type b. *Infect Immun* 1999;67:2503–14.
- Saul FA, Poljak RJ. Crystal structure of human immunoglobulin fragment Fab New refined at 2.0 angstrom resolution. *Protein Structure Function and Genetics* 1992;14:363–71.
- Celikel R, Varuguese KI. Crystal structure of the von Willibrand factor A1 domain in complex with the function blocking NMC-4Fab. *Nat Struct Biol* 1998;5:189–94.
- Lescar J, Stouracova R, Riottot M-M, *et al.* Three-dimensional structure of a Fab-peptide complex: structural basis of HIV-1 protease inhibition by a monoclonal antibody. *J Mol Biol* 1997;267:1207–22.
- Banfield MJ, King DJ, Mountain A, *et al.* VL:VH domain rotations in engineered antibodies: crystal structures of the Fab fragments from two murine anti-tumour antibodies and their engineered human constructs. *Protein Structure Function and Genetics* 1997;29:161–71.
- Topham CM, Srinivasan N, Thorpe CJ, *et al.* Comparative modelling of major house dust mite allergen Der p 1: structure validation using an extended environmental amino acid propensity table. *Protein Eng* 1994;7:869–94.
- Ghiara JB, Stura EA, Stanfield RL, *et al.* Crystal structure of the principal neutralization site of HIV-1. *Science* 1994;264:82–5.
- Stanfield RL, Takimoto-Kamimura M, Rini J, *et al.* Major antigen-induced domain rearrangements in an antibody. *Structure* 1993;1:83–93.
- Evans SV, Sigurskjold BW, Jennings HJ, *et al.* Evidence for the extended helical nature of polysaccharide epitopes. The 2.8 Å resolution structure and thermodynamics of ligand binding of an antigen binding fragment specific for alpha-(2 → 8)-polysialic acid. *Biochemistry* 1995;34:6737–44.
- Lescar J, Pellegrini M, Souchon H, *et al.* Crystal structure of a cross-reaction complex between Fab F9.13.7 and guinea fowl lysozyme. *J Biol Chem* 1995;270:18067–76.
- Clothia C, Lesk AM, Tramontano A, *et al.* Conformation of immunoglobulin hypervariable regions. *Nature* 1989;342:877–83.
- Clothia C, Lesk AM. Canonical structures for the hypervariable regions of immunoglobulins. *J Mol Biol* 1987;196:901–17.
- Tramontano A, Clothia C, Lesk AM. Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins. *J Mol Biol* 1990;215:175–82.

- 40 Sheriff S, Silverton EW, Padlan EA, *et al.* Three-dimensional structure of an antibody-antigen complex. *Proc Nat Acad Sci U S A* 1987;**84**:8075-9.
- 41 Colman PM, Laver WG, Varghese JN, *et al.* Three-dimensional structure of a complex of antibody with influenza virus neuraminidase. *Nature* 1987;**326**:358-63.
- 42 Satow Y, Cohen GH, Padlan EA, *et al.* Phosphocholine binding immunoglobulin Fab McPC603. An x-ray diffraction study at 2.7 Å. *J Mol Biol* 1986;**190**:593-7.
- 43 Shirai H, Kidera A, Nakamura H. Structural classification of CDR-H3 in antibodies. *FEBS Lett* 1996;**399**:1-8.
- 44 Schroeder HW, Jr, Hilson JL, Perlmutter RM. Structure and evolution of mammalian VH families. *Int Immunol* 1990;**2**: 41-50.
- 45 Daeron M, Malbec O, Latour S, *et al.* Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. *J Clin Invest* 1995;**95**: 577-85.
- 46 Takai T, Ono M, Hikida M, *et al.* Augmented humoral and anaphylactic responses in FcγRII-deficient mice. *Nature* 1996;**379**:346-9.