

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

J. E. McELVEEN, M. R. CLARK*, S. J. SMITH, H. F. SEWELL and F. SHAKIB

Division of Molecular & Clinical Immunology, University of Nottingham, Queen's Medical Centre, Nottingham and

*Division of Immunology, University of Cambridge, Cambridge, UK

Summary

Background Der p 1, a major mite allergen, elicits IgE antibody responses in 80% of patients suffering from dust mite allergy. Given the potent IgE eliciting properties of Der p 1, there is considerable interest in studying the molecular architecture of the variable (Fv) region of IgE antibodies specific for this allergen.

Objectives IgE is present in human serum at extremely low concentrations, and as such it is practically impossible to purify sufficient quantities for structural studies. We have therefore sought to sequence and model a representative murine monoclonal (MoAb) anti-Der p 1 antibody, as a surrogate human IgE.

Methods The cDNA coding for the Fv region of an anti-Der p 1 MoAb (2C7), that mimics the binding of human IgE to Der p 1, was amplified by PCR, cloned and sequenced. The predicted amino acid sequences were then compared with a directory of human germline V-gene segments. Modelling of the Fv region of MoAb 2C7 was carried out using the extensive database of existing immunoglobulin structures in the Brookhaven PDB.

Results The MoAb 2C7 heavy chain showed greater than 70% homology with three members of the V_H3 family, DP-35, DP-53 and DP-54. Similarly, the light chain showed greater than 70% homology with 11 V_K sequences, including the V_KII sequences DPK18, DPK19 and DPK28. A molecular model of the Fv region of MoAb 2C7 was generated and can be accessed from the EMBL databank.

Conclusions Antibodies similar to MoAb 2C7 could be generated as part of the human repertoire. The availability of 3-dimensional model of MoAb 2C7, as a surrogate human IgE antibody, combined with further data on its epitope specificity, will facilitate studies into IgE antibody responses to Der p 1.

Keywords: Der p 1, dust mite, IgE

Clinical and Experimental Allergy, Vol. 28, pp. 1427–1434. Submitted 1 December 1997; revised 4 February 1998; accepted 21 February 1998.

Introduction

The dust mite *Dermatophagoides pteronyssinus* is a major cause of allergic disease in the western world. It is estimated that about 10–20% of the population have IgE-mediated hypersensitivity to this mite species. More than 80% of individuals who are sensitive to *D. pteronyssinus* produce

IgE antibodies to Der p 1 [1,2], one of several major mite allergens, which can account for over 50% of the total anti-mite allergen response [3,4].

Given the potent IgE eliciting properties of Der p 1, there is considerable interest in studying the molecular architecture of the variable (Fv) region of IgE antibodies specific for this allergen. Such knowledge will be useful for elucidating the molecular interaction between IgE and Der p 1, as a basis for designing effective intervention strategies. However, IgE is present in human serum at extremely low

Correspondence: Dr F. Shakib, Division of Molecular & Clinical Immunology, Faculty of Medicine and Health Sciences, Queen's Medical Centre, Nottingham, NG7 2UH, UK.

concentrations (less than 1 µg/mL compared with approximately 10 mg/mL for IgG), and as such it is practically impossible to purify sufficient quantities for structural studies. Clearly, an alternative approach would be to use, as surrogate human IgE antibody, a representative murine monoclonal (MoAb) anti-Der p 1 antibody.

Although several MoAbs have been raised against Der p 1 [5,6], the majority of these antibodies do not inhibit the binding of human IgE to Der p 1, thereby indicating that they recognize different epitopes or are of much lower affinity than human IgE anti-Der p 1. Here, we describe the primary sequence of the Fv region of an anti-Der p 1 MoAb (2C7) that mimics the binding of human IgE to Der p 1. Furthermore, given that human IgE recognizes epitopes on Der p 1 that are discontinuous in nature [7–10], we have modelled the Fv region of MoAb 2C7 to facilitate further studies into how human IgE interacts with Der p 1, for which a model is available [11].

Materials and methods

Der p 1 preparation

Der p 1 was purified from lyophilized house dust mite culture supernatant (SmithKline Beecham Pharmaceuticals, Worthing, UK), by affinity chromatography, using anti-Der p 1 MoAb (4C1, Indoor Biotechnologies, Clwyd, UK) as described elsewhere [12]. The purity of the preparation was assessed by silver-stained SDS-12% PAGE analysis, and its identity confirmed by N-terminal sequencing on an automatic amino acid sequencer (PE Applied Biosystems, Foster City, CA, USA): the sequence obtained (TNAC-SINGNA) matched that of Der p 1 [13].

Monoclonal anti-Der p 1 antibodies

Antibodies 5H8 and 4C1 were obtained from Indoor Biotechnologies Limited. Antibody 2C7 (IgG2bκ) was produced by conventional hybridoma technology [14] by fusing spleen cells from an immunized Balb/c mouse with P3/NSO myeloma cells. The mouse was immunized with house dust mite (HDM) extract in complete Freund's adjuvant (200 µg, i.p.) followed, 85 days later, by a second injection of HDM extract in incomplete Freund's adjuvant (50 µg, i.v.). Hybridoma cells were selected by screening for reactivity with HDM extract and purified MoAb was tested against affinity purified Der p 1.

ELISA inhibition

Der p 1 (1 µg/mL) was bound to microtitre plates (Nunc Maxisorp, Life Technologies Limited, Paisley, UK) by capturing with anti-Der p 1 MoAb 5H8 (2.5 µg/mL). The

ability of MoAbs 4C1 and 2C7 to competitively inhibit the binding of biotinylated MoAb 2C7 to Der p 1 was tested using alkaline phosphatase-conjugated ExtrAvidin (Sigma Chemical Company, Poole, UK). Similarly, inhibition of the binding of IgE anti-Der p 1, from sera of dust mite allergic individuals (kindly provided by Dr Eric Saihan, Queen's Medical Centre, Nottingham, UK), by MoAbs 4C1 and 2C7 was assayed using alkaline phosphatase conjugated goat anti-human IgE (Sigma Chemical Company).

Reverse transcription and PCR

Approximately 1×10^6 2C7 hybridoma cells were harvested by centrifugation and homogenized using a QIASHRED-DER™ column (Qiagen Ltd, Dorking, UK). Total RNA was prepared using the Qiagen RNeasy kit according to manufacturers instructions. cDNA synthesis was performed by heating 10 µL of the total RNA preparation to 70°C for 5 min, then placing it on ice and adding 4 µL 5X Superscript buffer, 0.4 µL oligo(dT) primer (0.5 mg/mL, Pharmacia, Uppsala, Sweden), 2 µL dNTP (5 mmol/L), 2 µL dithiothreitol (0.1 mol/L), 0.6 µL RNasin™ ribonuclease inhibitor (40 unit/mL, Promega Corporation, Madison, WI, USA) and 200 unit Superscript II™ Rnase H⁻ reverse transcriptase (Gibco BRL, Middlesex, UK). This was followed by incubation at 42°C for 1 h.

PCR was performed as described [15], using 1 µL of cDNA in each reaction and the primers given in Table 1 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 µg/mL) and the DNA was visualized using an ultraviolet transilluminator. Molecular weight markers were a 100 base pair DNA ladder (Promega).

Cloning PCR products

Ligation was performed using a TA cloning kit (Invitrogen, San Diego, CA, USA) with the pCR™ 2.1 plasmid. Transformation of INVαF' One Shot™ 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 µg/mL respectively), using QIA tip 20 columns (Qiagen Ltd). 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 PRISM™ 310 Genetic Analyzer, using the ABI PRISM™

Table 1. PCR primers [15] used to amplify mouse antibody Fd fragments (a) and complete kappa chains (b). Restriction sites are underlined.

Name	Primer
(a)	
V _H fr1a	5'-GTAAGACGTCC/AAGCTTCAGGAGTCA/GGGACC-3'
V _H fr1b	5'-GTAAGACGTCCAGCTGAAGG/CAG/CTCAGGACC-3'
V _H fr1c	5'-ATATGACGTCA/CT/AGC/GT/GGGTGGAGTCTGGGGGA-3'
V _H fr1d	5'-ATATGACGTCAA/GG/CC/GTGGT/AGGAATCTGGAGGA-3'
V _H fr1e	5'-ATATGACGTCAA/GGC/GTGG/AGT/GGAGTCTGGAGG-3'
V _H fr1f	5'-AATTGACGTCCAG/AC/GT/CGCAGCAG/AC/TCTGGG-3'
V _H fr1g	5'-AATTGACGTCCAGC/TTGC/GA/TGCAG/ATCTGGA-3'
V _H fr1h	5'-AATTGACGTCCAGCTGCAGCAGTCT/AGTG-3'
V _H fr1i	5'-AATTGACGTCC/AAG/CC/TTGC/GT/AGCA/TGA/TCTGGAGG-3'
V _H fr1j	5'-AATTGACGTCCAGC/ATG/CCAGCAGC/TCTGG-3'
Reverse	5'-ATTAAGTCGACCT/GT/CGGTG/CC/TTGCTGGCC/TGGGTG-3'
Gamma	
(b)	
V _K fr1a	5'-ATATCCATGGCAGACG/ATCC/AAGATG/AAT/CCCAGT/ACTC/ACA-3'
V _K fr1b	5'-ATATCCATGGCAG/AAC/AATTG/TTGCTGACT/CCAG/ATC/TTCC-3'
V _K fr1c	5'-ATATCCATGGCAGATG/AT/CTG/TTGATGACCCAACTCCA-3'
V _K fr1d	5'-ATATCCATGGCAC/GA/GAAT/ATG/CTTCTCA/TC/ACAGTCTCC-3'
V _K fr1e	5'-ATATCCATGGCAG/AA/GCA/GTTG/CT/AGATGA/TCACAGTCT/GCCA-3'
V _K fr1f	5'-ATATCCATGGCAGATATTGTGATG/AACG/TCAGGC/ATG/AC/AA-3'
V _K fr1g	5'-ATATCCATGGCAG/AA/GC/TATTGTGATGACCCAG/AT/ACT/AC-3'
Reverse	5'-CTACCTCGAGTTAACACTCATTCTGTTGAAGC-3'
Kappa	

Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). Sequences from different clones were aligned and translated using the program SeqApp (D. Gilbert, Indiana University, Bloomington, IN, USA) and compared with the Kabat database [16] to identify any errors arising from the misincorporation of bases during PCR.

Sequence analysis and molecular modelling

Database searching and sequence analysis were performed using the University of Cambridge School of Biological Sciences computing facilities and the Wisconsin Package Version 9.0 Genetics Computer Group (GCG) program packages. The V-BASE directory [17] of human immunoglobulin heavy and light chain segments was downloaded (V-BASE Sequence Directory, Tomlinson *et al.*, MRC Centre for Protein Engineering, Cambridge, UK, Release 1.0, 7 March 1997) and prepared for use with the GCG sequence analysis package. Comparisons of the MoAb 2C7 protein sequence with the nucleotide sequences in the V-BASE directory were carried out using the program 'TFASTA' [18], and automatic alignments of the most homologous sequences were produced. Molecular modelling of the MoAb 2C7 sequences was carried out using the programs QUANTA (version 96.0516) and

MODELER (Molecular Simulations Incorporated, Burlington, MA, USA). These programs were run on a Silicon Graphics Iris Indigo workstation running the IRIX 5.3 operating system.

Results

Specificity of MoAb 2C7 relative to human IgE

We have first established that MoAb 4C1, previously shown to partially inhibit the binding of human IgE to Der p 1 [5], also inhibits the binding of MoAb 2C7 to Der p1 (Fig. 1a), thereby indicating similar epitope specificity. To further confirm that MoAb 2C7 recognizes the same epitope on Der p 1 as human IgE, inhibition experiments were performed with sera from five patients with high levels of IgE anti-Der p 1. Results demonstrate that MoAb 2C7 inhibits the binding of human IgE anti-Der p 1 to a greater extent (up to 70%) compared with MoAb 4C1 (up to 40%) (Fig. 1b).

PCR products and cloning

PCR amplification of cDNA using the heavy chain primers resulted in a single band of approximately 650 bp with the

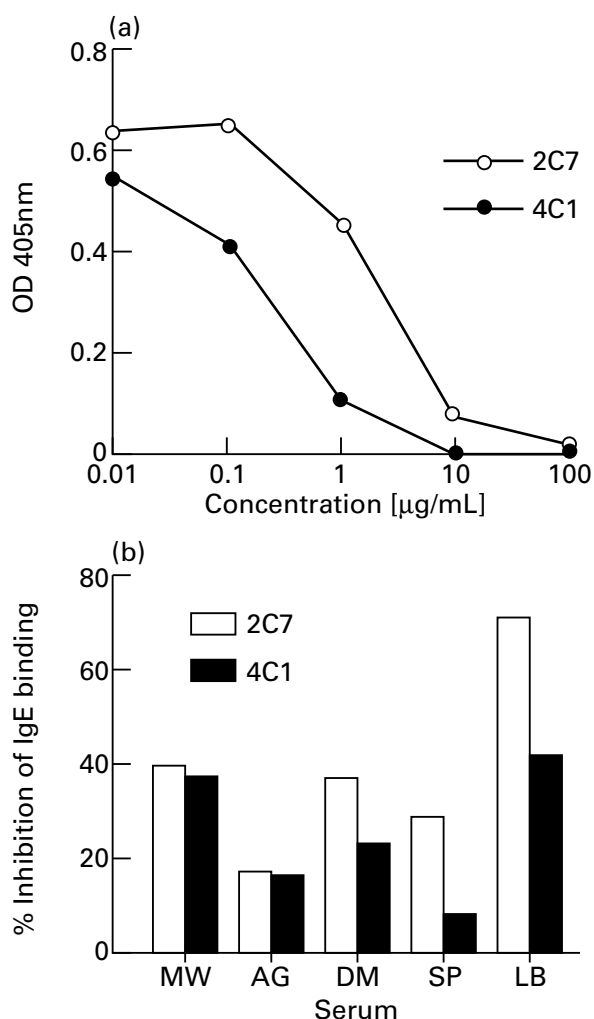


Fig. 1. Ability of MoAbs 2C7 (open circles or bars) and 4C1 (filled circles or bars) to competitively inhibit the binding of biotinylated MoAb 2C7 (a) and human IgE (in sera obtained from dust mite allergic individuals MW, AG, DM, SP and LB) (b) to immobilized Der p 1. Binding of biotinylated MoAb 2C7 and human IgE were detected using extravidin and alkaline phosphatase-conjugated goat anti-human IgE, respectively. IgE anti-Der p 1 containing serum samples were diluted 1/8 to 1/64 to render them equipotent (i.e. giving optical density values between 0.5–0.6).

primer V_Hfr1e, which is the expected size for the Fd fragment of the heavy chain. The κ chain primer V_kfr1c also produced a band of 650 bp when used in conjunction with the reverse κ primer. 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 2 shows the results of this analysis for five representative clones for the heavy chain and five representative clones for the κ chain, together with the corresponding PCR products.

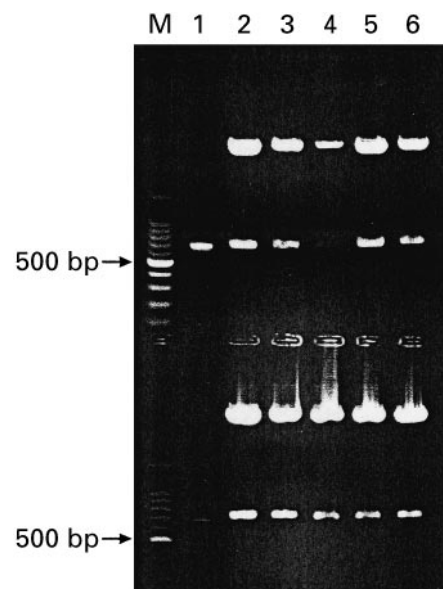


Fig. 2. A 1% agarose gel showing PCR products and restriction digests of plasmids containing the cDNA inserts for the heavy chain Fd fragment (top lanes 2–6) and the κ chain (bottom lanes 2–6). Lane 1 contains the corresponding PCR products and lane M is a 100 bp ladder.

Sequence analysis

The protein sequences for the light chain and the heavy chain of MoAb 2C7 are given in Fig. 3. These represent the sequences of individual clones which were identified as containing no PCR errors by comparison with other clones and the Kabat database. The first eight amino acids of the light chain and the first nine amino acids of the heavy chain are determined by the degenerate PCR primers.

Homology of the MoAb 2C7 with human immunoglobulin V-gene segments

The MoAb 2C7 heavy and light chain V-region protein sequences were compared with the V-BASE nucleotide sequences, and a number of homologous V-genes were identified (Fig. 3). The MoAb 2C7 heavy chain showed greater than 70% homology with three members of the V_H3 family, DP-35, DP-53 and DP-54 [19]. This homology also included the predicted CDR1 and CDR2 regions, and many of the amino acid changes found represent conservative substitutions. Similarly, the light chain showed greater than 70% homology with 11 V_K sequences, including the V_KII sequences DPK18, DPK19 and DPK28 [20].

A model of the Fv region of MoAb 2C7

A predicted three-dimensional structure of MoAb 2C7 Fv

	10	20	30	40	50	60	70	80	90	98											
2C7VH	DVKVVE	SGGGLV	QPGGSL	KL	SCTASG	FD	FSRYW	MSWVR	QAPGK	GLEWIG	EINPDG	SPIN	YTPSL	KDKFI	ISRD	NSKNTLYL	QISK	VRSE	D	TALY	YCAR
DP-35	Q-QL	-----	K-----	R---	A---	T--	D-Y--	I-----	VS	Y-SSS--	T-Y-AD	V-GR-T---	A--S---	MNSL	A---	V-----					
DP-53	E-QL	-----	R-----	A---	T--	S--	H-----	V-VSR--	S---STS	AD-V-GR	T-----	A-----	MNSL	A---	V-----						
DP-54	E-QL	-----	R---A---	T--S-----	-----	VAN-KQ--	EKY-VD	V-GR-T---	A--S---	MNSL	A---	V-----									

	10	20	30	40	50	60	70	80	90	100									
2C7VL	DVLMTQ	TPLSLP	VSFGDQ	VSISCR	SSQSLANS	YGNTYLS	WYLHKP	QGSPQL	LIYGIS	DRFSGV	PDRFSG	SGSG	SGTD	FTLKIS	TIKPED	LGMYYC	LQ	GTHQ	P
DPK18	--V--S	-----	TL-QPA	-----	VY-D---	N-FQQR	-----	RR--KV	N-----	-----	RVEA	--V--M	-----	W-					
DPK19	--V--S	-----	TL-QPA	-----	VY-D---	N-FQQR	-----	RR--KV	NWD-----	-----	RVEA	--V--M	-----	W-					
DPK28	-IM-----	S-TP-QPA	-----	K-----	LH-D-K--	Y--Q-----	-----	EV-S-----	-----	-----	RVEA	--V--M	-----	I-L-					

Fig. 3. Protein sequence alignments of the MoAb 2C7 heavy chain V-region (2C7VH) and light chain V-region (2C7VL) with predicted human protein sequences obtained from the V-BASE directory. Three human V_{HIII} family sequences, DP-35, DP-53 and DP-54 [19], and three human V_{KII} family sequences, DPK18, DPK19 and DPK28 [20], are aligned.

region was constructed by modelling on sequence-homologous structures within the Brookhaven Protein Data Bank (PDB) [21]. A search of the PDB database was performed using the BLAST network service at NCBI, and this produced a number of structures with reasonable

homology to the MoAb 2C7 heavy chain, light chain or both. Four structures, 1igc [22], 1igf [23], 1jel [24] and 4fab [25], which had high homology with both chains were selected (Fig. 4). The model was built using the program MODELER [26] through an interface in the QUANTA

2C7VH	DVKVVE	SGGGLV	QPGGSL	KL	SCTASG	FD	FSRYW	MSWVR	QAPGK	GLEWIG	EINPDG	SPIN	. .	YTPSL	KDKFI	ISRD
1igc	--QL	-----	R---	A---	T--SFG	H-----	E-----	VAY-SSGS	TLH. .	ADTV	GR-T---					
1igf	E-QL	-----	K-----	A---	T--CA	-----	T-E-R--	VAG-SSG	.SY.TF	PDTV	GR-----	N				
1jel	Q-QLQQ	--PE--	R--S-V-I--	KG--YT	TT-A-H--	K-SHA	S-----	L-SPSS	.GYTS.	NGEF	G-ATMTVD					
4fab	E--LD-T	-----	RPM---	V----	T--D-W-N	-----	S-E-----	VAQ-RNK	PYNYETY	SDSV	GRFT----					

2C7VH	NSKNTLYL	QISKVR	SEDTAL	YYCAR	.PGRLLL	HFDYWG	QGTTLT	VSSAKT	TPPS	
1igc	-P----	F--MTSL	-----	GM-----	WGNYP	PYYAM	-----	SV-----		
1igf	-AR---	S--M-SL	-----	I--T-YSSD	PFY.	-----				
1jel	K-SS-A	MELARLT	---	S-I-----	VM-EQYF	. .	V--A--	V-----	A-----	
4fab	D--SSV---	MNNL-V--	MGI---	T. . .	GSYYGM	-----	SV-----	A--		

2C7VL	DVLMTQ	TPLSLP	VSFGDQ	VSISCR	SSQSLANS	YGNTYLS	WYLHKP	QGSPQL	LIYGIS	DRFSGV	PDRFSG	SGSG	SGTD		
1igc	NIV---	S-K-MSM	V-ER-TLT	KA-ENVV		-YV--	QQ--E--	K----	A-N-YT	-----	T----	A--			
1igf	-----	L--A-----	N-TILL	D-D--E--	Q-----	K--KV	N-----								
1jel	-----	L--A-----	IVHGN	-----	E--Q-----	K--S--S	-----								
4fab	--V-----	L--A-----	VH-Q----	R--Q-----	KV--KV	N-----									

2C7VL	FTLKISTIK	PEDLGM	YYCLQG	THQPWT	FGGGTK	LEIKRADA	AAPT	VSIF	PPSSEQ	
1igc	---T--	SVQA--	AD-H-G--	NSY-Y	-----					
1igf	-----	RVEA---	V--F--S-V	P-----						
1jel	-----	RVQA---	V--F--S-V	Y-----						
4fab	-----	RVEA---	V-F-S-S-V	-----						

Fig. 4. Protein sequence alignments of the MoAb 2C7 heavy chain V-region (2C7VH) and light chain V-region (2C7VL) with four homologous sequences (1igc, 1igf, 1jel and 4fab) which have structural coordinates available within the Brookhaven PDB. These structures were used as the basis for homology modelling of the Fv region of MoAb 2C7. CDR regions are indicated by solid lines above the sequences; dots indicate missing amino acids.

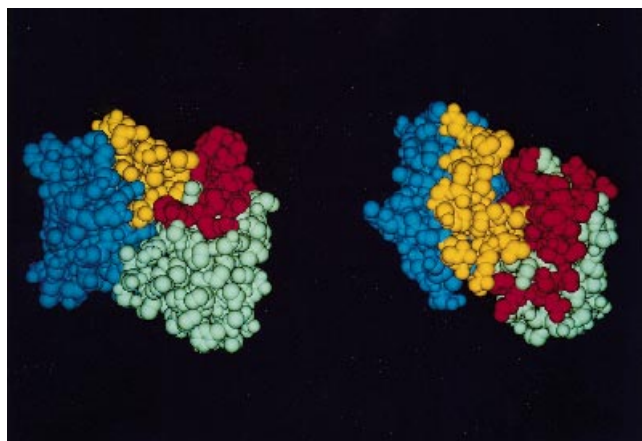


Fig. 5. A molecular model of the Fv region of MoAb 2C7, showing a side view (left) and a top view (right). The heavy chain and the light chain are shown in blue and green, respectively, and the CDRs of the heavy chain and the light chain are shown in orange and red, respectively.

package running on a Silicon Graphics Iris Indigo workstation. The MoAb 2C7 sequences were aligned and superimposed with the four structures, and the model was produced. During subsequent refinements, the 1igc structure was eliminated from the comparison because it had CDR loops of a different length to the MoAb 2C7 antibody. As might be expected from the sequence homologies apparent in Fig. 4, the predicted light chain model very closely resembles the three structures. Several refinements of the model were made. In the final model (Fig. 5) there were very few restraint violations greater than 5.0, particularly for the Framework regions which are mainly made up of β -strands. The α -carbon backbone structures for all the models showed an excellent agreement except for the C' strand of the heavy chain. As expected, in a comparison of the different models, it was found that the major uncertainties were in the orientations of the side chains particularly for the CDR3 loops of the heavy and light chains.

Discussion

Der p 1 elicits IgE antibody responses in 80% of patients suffering from dust mite allergy [1,2]. From a therapeutic point of view, there is therefore considerable interest in elucidating the nature of the molecular interaction between IgE and Der p 1, as a basis for designing effective intervention strategies. Given that IgE is present in trace quantities in serum, it is practically impossible to purify sufficient quantities for structural studies. We have therefore sought to obtain a mouse monoclonal antibody which might mimic the properties of a major component of the human IgE response to

Der p 1. The MoAb 2C7 described here and briefly elsewhere [27,28] fits this description. In ELISA, MoAb 2C7 is capable of inhibiting a substantial component (up to 70%) of the human IgE response to Der p 1. In this respect, it performs better than MoAb 4C1, which has also been reported to share an epitope specificity with human IgE anti-Der p 1 [5]. It is clearly difficult to ascertain that the inhibitory effects of these monoclonal antibodies are due to competition for the same epitope, rather than to steric hindrance. However, a range of other monoclonal antibodies to Der p 1 were previously tested and showed little or no inhibition [5]. These observations prompted us to carry out structural studies of the Fv region of MoAb 2C7, as a prototype human IgE anti-Der p 1.

The MoAb 2C7 immunoglobulin heavy and light chain V-regions were cloned and sequenced. This showed that MoAb 2C7 uses a germline gene from the mouse V_H4 (X-24) family, which shares considerable homology with the human V_H3 gene family [29]. The predicted amino acid sequences were then compared with a directory of human germline V-gene segments, and not surprisingly homologous human V_H and V_K gene segments were identified. The MoAb 2C7 heavy chain showed greater than 70% homology with three members of the V_H3 family, DP-35, DP-53 and DP-54 [19]. Similarly, the light chain showed greater than 70% homology with 11 V_K sequences, including the V_{KII} sequences DPK18, DPK19 and DPK28 [20]. The V-BASE directory contains gene segments which are commonly found within the human population [17,19,20]. It is therefore likely that antibodies similar to MoAb 2C7 could be generated as part of the human repertoire, particularly when allowing for combinatorial effects and also somatic mutations of the CDR regions within the rearranged genes. This is, clearly, in keeping with our data showing similarity in epitope recognition between MoAb 2C7 and human IgE anti-Der p 1.

The structure of the Fv region of MoAb 2C7 was modelled using the extensive database of existing immunoglobulin structures in the Brookhaven PDB [21]. As expected, the search revealed a large number of structures with sequence homology to the MoAb 2C7 heavy chain, light chain or both. From these structures, four were selected on the basis that they had high sequence homology with both chains. Several rounds of modelling were carried out involving refinements of the sequence alignments with the existing structures, particularly with regard to the lengths and alignments of the CDR3 loops. The models generated were examined for restraint violations and for any unusual features, such as exposed hydrophobic or buried hydrophilic or polar side chains, and a final model was selected. The selected model can be accessed (accession number AJ001811) from the EMBL databank, where it should be a useful resource, particularly when combined with the published model of Der p 1 [11].

Having cloned and sequenced the Fv region of MoAb 2C7, it will now be relatively straightforward to produce a chimaeric human IgE construct of it. This will provide a useful tool to study the molecular interactions involved in IgE-mediated responses. The availability of three-dimensional models of MoAb 2C7 and Der p 1 [11], combined with further data on the epitope specificity of MoAb 2C7, will also facilitate studies into IgE antibody responses to this important allergen. Such efforts may lead to new ways of suppressing Der p 1-specific IgE synthesis and effector functions by, for instance, anti-idiotypic antibodies [30].

Acknowledgements

The authors wish to acknowledge the financial support of the BBSRC (Grant Reference T03598). For enquiries concerning the coordinates of the molecular model of 2C7 please contact M. Clark (e-mail: mrc7@cam.ac.uk).

References

- Chapman MD, Rowntree SR, Di Prisco DeFuenmajor MS et al. Quantitative assessments of IgG and IgE antibodies to inhalant allergens in patients with atopic dermatitis. *J Allergy Clin Immunol* 1983; 72:27–33.
- Krill S, Baldo BA, Sutton R, Basten A. Antigens and allergens from the common house dust mite *Dermatophagoides pteronyssinus*. I. Demonstration of multiple allergens by immunochemical and biologic analysis. *J Allergy Clin Immunol* 1984; 74:132–41.
- Chapman MD, Platts-Mills TAE. Purification and characterisation of the major allergen from *Dermatophagoides pteronyssinus*—antigen P1. *J Immunol* 1980; 125:587–92.
- van der Zee JS, van Swieten P, Jansen HM, Aalberse RC. Skin tests and histamine release with P1-depleted *Dermatophagoides pteronyssinus* body extracts and purified P1. *J Allergy Clin Immunol* 1988; 81:884–96.
- Chapman MD, Heymann PW, Platts-Mills TAE. Epitope mapping of two major inhalant allergens, Der p 1 and Der f 1 from mites of the genus *Dermatophagoides*. *J Immunol* 1987; 139:1479–84.
- Lind P, Hansen OC, Horn N. The binding of mouse hybridoma and human IgE antibodies to the faecal allergen Der p 1 of *Dermatophagoides pteronyssinus*. *J Immunol* 1988; 140:4256–62.
- Greene WK, Cyster JG, Chua KY et al. IgE and IgG binding of peptides expressed from fragments of cDNA encoding the major house dust mite allergen Der p 1. *J Immunol* 1991; 147:3768–73.
- Greene WK, Thomas WR. IgE binding structures of the major house dust mite allergen Der p 1. *Mol Immunol* 1992; 29:257–62.
- Jeannin P, Delneste Y, Buisine E et al. Immunogenicity and antigenicity of synthetic peptides derived from the mite allergen Der p 1. *Mol Immunol* 1993; 30:1511–18.
- Collins SP, Ball G, Vonarx E et al. Absence of continuous epitopes in the house dust mite major allergens Der p 1 from *Dermatophagoides pteronyssinus* and Der f 1 from *Dermatophagoides farinae*. *Clin Exp Allergy* 1996; 26:36–42.
- 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 Engng* 1994; 7:869–94.
- Lombardero M, Heymann PW, Platts-Mills TAE et al. Conformational stability of B cell epitopes on Group I and Group II *Dermatophagoides* spp. allergens. *J Immunol* 1990; 144:1353–60.
- Chua KY, Stewart GA, Thomas WR et al. Sequence analysis of cDNA coding for a major house dust mite allergen, Der p 1. Homology with cysteine proteases. *J Exp Med* 1988; 167:175–82.
- Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975; 256:495–97.
- Kettleborough CA, Saldanha J, Ansell KH, Bendig MM. Optimization of primers for cloning libraries of mouse immunoglobulin genes using the polymerase chain reaction. *Eur J Immunol* 1993; 23:206–11.
- Kabat EA, Wu TT, Reid-Miller M et al. Sequences of proteins of immunological interest. 5th Edn. US Department of Health & Human Services. Bethesda, 1991.
- Cooke GP, Tomlinson IM. The human immunoglobulin V_H repertoire. *Immunol Today* 1995; 16:237–42.
- Pearson WR, Lipman DJ. Improved tools for biological sequence comparison. *Proc Natl Acad Sci USA* 1988; 85:2444–48.
- Tomlinson IM, Walter G, Marks JD et al. The repertoire of human germ line V_H segments reveals about fifty groups of V_H segments with different hypervariable loops. *J Mol Biol* 1992; 227:776–98.
- Cox JPL, Tomlinson IM, Winter G. A directory of human germ line V_k segments reveals a strong bias in the usage. *Eur J Immunol* 1994; 24:827–36.
- Bernstein FC, Koetzle TF, Williams GJB et al. The Protein Data Bank: A Computer-based Archival File for Macromolecular Structures. *J Mol Biol* 1977; 112:535–42.
- 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.
- Stanfield RL, Fieser TM, Lerner RA, Wilson IA. Crystal structures of an antibody to a peptide and its complex with peptide antigen at 2.8 angstrom. *Science* 1990; 248:712–19.
- Prasad L, Sharma S, Vandonselaar M et al. Evaluation of mutagenesis for epitope mapping: structure of an antibody-protein antigen complex. *J Biol Chem* 1993; 268:10705–8.
- Herron JN, He X, Mason ML et al. Three-dimensional structure of a Fluorescein-Fab complex crystallised in 2-Methyl-2, 4-Pentanediol. *Prot Struct Funct Genet* 1989; 5:271–80.
- Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. *J Mol Biol* 1993; 234:779–815.
- Austin EA, Robins RA, Powell-Richards A et al. Characterisation of anti-allergen and anti-idiotypic antibodies for potential therapy of allergic disease. *Clin Exp Allergy* 1994; 24:189.

- 28 Byers VS, Baldwin RW, Ma Y, Price MR. Epitopes for human IgE on Der p I probed using murine monoclonal antibodies. *J Allergy Clin Immunol* 1997; 99:S436.
- 29 Haino M, Hayashida H, Miyata T et al. Comparison and evaluation of human immunoglobulin V_H segments located in the 3' 0.8-kilobase region. *J Biol Chem* 1994; 269:2619.
- 30 Shakib F. Editorial: Controlling allergy with anti-idiotypic antibodies. *Clin Exp Allergy* 1995; 23:908–9.