

# Characterisation of a mouse monoclonal anti-idiotypic reactive with a V region sequence commonly used by human immunoglobulins

J E McElveen, P B Furtado, S J Smith, M R Clark, I Spendlove, H F Sewell, F Shakib

## Abstract

**Background**—A mouse monoclonal antibody (2C7/IgG2bκ) has been described recently, which is directed against the major house dust allergen Der p 1, and whose epitope specificity is representative of a major component of the human IgE anti-Der p 1 response.

**Aims**—To characterise an anti-idiotypic antibody (2G10/IgG1κ) raised against monoclonal antibody 2C7 as surrogate human IgE anti-Der p 1.

**Methods**—The specificity of the anti-idiotypic antibody 2G10 was determined by competitive inhibition experiments using human and mouse immunoglobulins of known V<sub>H</sub> gene families. The epitope recognised by monoclonal antibody 2G10 was located on the molecular model of the Fv (fragment variable) region of monoclonal antibody 2C7.

**Results**—The data suggest that monoclonal antibody 2G10 is directed against a crossreactive idiotype on human IgE that is shared by polyclonal IgG. Competitive inhibition studies against human immunoglobulins, representative of V<sub>H</sub>2, V<sub>H</sub>3, and V<sub>H</sub>4 gene families, showed that monoclonal antibody 2G10 is mostly likely to be directed against sequences encoded by either V<sub>H</sub>3 or V<sub>H</sub>4 genes. The fact that monoclonal antibody 2G10 binds to the humanised (complementarity determining region (CDR) grafted) CAMPATH-1H antibody, but not to the original rat CAMPATH-1 YTH34.5.6 antibody, indicates that it is directed against a framework region rather than the CDRs. Analysis of amino acids in the V<sub>H</sub> region for charge, hydrophobicity, and accessibility suggests that reactivity with monoclonal antibody 2G10 is defined by a hexapeptide spanning residues 74–79 within framework region 3.

**Conclusion**—The anti-idiotypic monoclonal antibody 2G10 could potentially be used as a probe for determining the contribution of the V<sub>H</sub>3 and V<sub>H</sub>4 gene segments to antigenic specificity.

(J Clin Pathol: Mol Pathol 2000;53:77–82)

Keywords: antibody; anti-idiotypic; Der p 1; IgE; mite

We have recently cloned, sequenced, and three dimensionally modelled the V regions of a mouse monoclonal antibody (2C7/IgG2bκ) directed against the major house dust mite

allergen Der p 1.<sup>1</sup> The predicted amino acid sequences were then compared with the V-BASE directory of human germline V gene segments<sup>2–4</sup> and homologous V<sub>H</sub> and V<sub>K</sub> gene segments were identified.<sup>1</sup> The monoclonal antibody 2C7 heavy chain showed greater than 70% homology with three members of the V<sub>H</sub>3 family: DP-35, DP-53, and DP-54.<sup>3</sup> Similarly, the light chain showed greater than 70% homology with 11 V<sub>K</sub> sequences, including the V<sub>K</sub>II sequences DPK18, DPK19, and DPK28.<sup>4</sup>

The V-BASE directory contains immunoglobulin gene segments that are commonly found within the human population.<sup>2–4</sup> Therefore, it is likely that antibodies similar to monoclonal antibody 2C7 could be generated as part of the human repertoire, particularly when allowing for combinatorial effects and also somatic mutations of the complementarity determining regions (CDRs) within the rearranged genes. This is very much in keeping with the results of our competition experiments, which showed that the epitope specificity of monoclonal antibody 2C7 is representative of a major component of the human IgE response to Der p 1.<sup>1</sup>

These observations prompted us to attempt to raise anti-idiotypic antibodies against monoclonal antibody 2C7, as surrogate human IgE anti-Der p 1, with a view to using them as probes for defining the specificity of human IgE anti-Der p 1, and as immunomodulatory agents to suppress the biosynthesis of such IgE antibodies. Here, we describe a mouse monoclonal anti-idiotypic antibody (2G10/IgG1κ) directed against monoclonal antibody 2C7, which apparently recognises a V region sequence commonly used by human immunoglobulins.

## Materials and methods

### ANTIBODY REAGENTS

Mouse monoclonal antibody 2C7 (IgG2bκ) was produced by conventional hybridoma technology, as described recently.<sup>1</sup> Other anti-Der p 1 monoclonal antibodies, namely 5H8 and 4C1,<sup>5</sup> were obtained from Indoor Biotechnologies Limited (Manchester, UK). Anti-human IgE monoclonal antibody (HE-47E)<sup>6</sup> was kindly provided by Dr S Harada (Shionogi Institute for Medical Science, Osaka, Japan). Chimaeric mouse–human IgE anti-NP<sup>7</sup> was obtained from Serotec (Oxford, UK). A human myeloma IgEλ (IgE-WT) was purified by affinity chromatography from a plasma sample kindly provided by Professor D Stanworth (Peptide Therapeutics plc, Cambridge, UK). A purified human myeloma IgG1κ protein

Division of Molecular and Clinical Immunology, University of Nottingham, Faculty of Medicine and Health Sciences, Queen's Medical Centre, Nottingham NG7 2UH, UK

J E McElveen  
P B Furtado  
S J Smith  
H F Sewell  
F Shakib

Immunology Division, Department of Pathology, Cambridge University, Tennis Court Road, Cambridge CB2 1QP, UK

M R Clark

CRC Department of Academic Oncology, University of Nottingham, Faculty of Medicine and Health Sciences, City Hospital, Nottingham NG5 1PB, UK  
I Spendlove

Correspondence to:  
Dr Shakib  
email: farouk.shakib@nottingham.ac.uk

Accepted for publication  
25 January 2000

(IgG-CH) was also obtained from Professor Stanworth. The human monoclonal antibody 105AD7 (IgG1 $\kappa$ ),<sup>8</sup> the rat CAMPATH-1 antibody YTH34.5.6 (IgG2 $\alpha$ ), and its humanised (CDR grafted) version, CAMPATH-1H (IgG1 $\kappa$ ), are described elsewhere.<sup>9</sup> The human monoclonal antibodies Cal-4G and A2 were kindly donated by Dr K Potter (University of Southampton, Southampton, UK). Cal-4G is an IgM $\lambda$  monoclonal antibody that uses the V<sub>H</sub>4–34 germline gene segment,<sup>10 11</sup> and A2 is a recombinant IgA $\kappa$  form of the human V<sub>H</sub>3 encoded antibody RF-SJ3(57K).<sup>12 13</sup> The polyclonal IgG antibodies IgG-MW and IgG-JM were isolated by protein G affinity chromatography from two normal human sera. The mouse monoclonal antibodies MOPC-21 (IgG1 $\kappa$ )<sup>14 15</sup> and MOPC-141 (IgG2b $\kappa$ )<sup>16 17</sup> were purchased from Sigma Chemical Company (Poole, Dorset, UK).

#### PRODUCTION OF MOUSE MONOCLONAL ANTI-IDIOTYPES

The anti-idiotype monoclonal antibodies 2G10 (IgG1 $\kappa$ ) and 4E10 (IgG1 $\kappa$ ) were produced by immunising mice with monoclonal antibody 2C7 coupled to keyhole limpet haemocyanin (2C7-KLH). The monoclonal antibody 2G10 was derived from a CBA mouse given an initial subcutaneous injection of 50  $\mu$ g 2C7-KLH in complete Freund's adjuvant (CFA), followed by three injections of 2C7-KLH in incomplete Freund's adjuvant (IFA; 50  $\mu$ g, intraperitoneally) at 14 day intervals, and a final injection of native 2C7 (50  $\mu$ g intravenously) 86 days later (that is, four days before fusion). The monoclonal antibody 4E10 was derived from a Balb/c mouse given an initial subcutaneous injection of 50  $\mu$ g 2C7-KLH in CFA with a booster intraperitoneal injection 21 days later, followed by a final intraperitoneal injection of 2C7 after 42 days (that is, five days before fusion). Spleen cells from monoclonal antibody 2C7 immunised mice were fused with P3/NSO myeloma cells, and hybridomas secreting anti-idiotype antibodies were selected by screening for reactivity with monoclonal antibody 2C7 in a "sandwich" enzyme linked immunosorbent assay (ELISA). Briefly, microtitre plates (Nunc Maxisorp, Life Technologies Limited, Paisley, UK) were coated with monoclonal antibody 2C7 (1  $\mu$ g/ml) and incubated with culture supernatant. Bound anti-idiotype antibody was detected by the addition of biotinylated monoclonal antibody 2C7, followed by alkaline phosphatase conjugated ExtrAvidin (Sigma Chemical Company). Secreting hybridomas were cloned three times by limiting dilution. The monoclonal antibodies were purified from spent culture supernatants by protein G affinity chromatography.

#### DETERMINATION OF THE SPECIFICITY OF MONOCLONAL ANTIBODIES 2G10 AND 4E10

The specificity of the anti-idiotype monoclonal antibodies 2G10 and 4E10 was assessed by testing them against a panel of 20 mouse monoclonal antibodies, including two IgG2b isotype controls, two other anti-Der p 1 monoclonal antibodies (5H8 and 4C1), and 16

monoclonal antibodies of irrelevant specificities, which were substituted for solid phase monoclonal antibody 2C7 in the sandwich ELISA described above. In another modification of the sandwich ELISA, the specificity of monoclonal antibody 2G10 was tested further by preincubation with a selection of mouse and human monoclonal or myeloma antibodies representative of different V<sub>H</sub> gene families before incubation on the monoclonal antibody 2C7 coated plate.

The reactivity of monoclonal antibodies 2G10 and 4E10 with human IgE was detected by capturing IgE on CHK1E1 cells (kind gift from Professor J-P Kinet, Harvard University, Boston, Massachusetts, USA) expressing the  $\alpha$  and  $\gamma$  subunits of the high affinity IgE receptor (Fc $\epsilon$ RI).<sup>18</sup> Briefly, the cells ( $2 \times 10^4$ /well) were cultured for three days at 37°C in 96 well plates in Iscove's modified Dulbecco's medium (IMDM) containing 10% fetal calf serum (FCS), L-glutamine, and Geneticin (Life Technologies Limited). All subsequent steps were performed at room temperature. The cells were washed gently three times with IMDM containing 2% FCS and 0.05% sodium azide (wash medium), and incubated for 30 minutes with either 1/4 dilution of human serum (18 in total; 12 atopic patients, with or without IgE anti-Der p 1, and six non-atopic controls) or 1  $\mu$ g/ml either of chimaeric mouse-human IgE anti-NP or human myeloma IgE $\lambda$  (IgE-WT). Cells were washed as before and monoclonal antibodies 2G10 or 4E10 were added at a concentration of 10  $\mu$ g/ml in wash medium. A mouse antihuman IgE monoclonal antibody (HE-47E) was used as control. The cells were incubated for one hour and washed as before. Bound monoclonal antibody was detected with a goat antimouse IgG conjugated to alkaline phosphatase (Sigma Chemical Company). Background values for wells containing no IgE were subtracted from the mean value for duplicate wells containing IgE. A modification of this assay was used to assess the ability of different antibody preparations to block the binding of monoclonal antibody 2G10 to CHK1E1 captured human IgE. The assay was carried out as described above, except that monoclonal antibody 2G10 was preincubated with the test antibody for 30 minutes.

#### SERUM IgE ASSAYS

Total IgE concentrations in serum were determined using a Milenia IgE kinetic EIA kit (DPC, Oxford, UK). Der p 1 specific IgE was determined by ELISA, as described recently.<sup>1</sup>

#### SEQUENCE ALIGNMENT AND MOLECULAR MODELLING

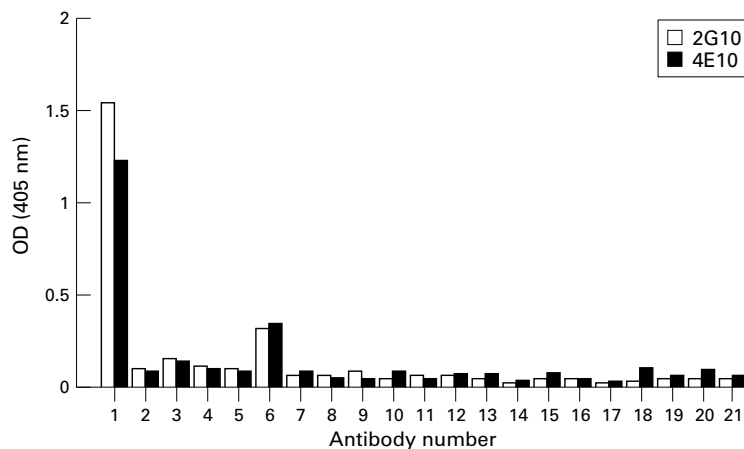
Comparison of the monoclonal antibody 2C7 protein sequence with the sequences of antibodies tested for reactivity with monoclonal antibody 2G10 was carried out using Gene Doc (Nicholas KB, Nicholas HBJr, 1997, Gene Doc: analysis and visualisation of genetic variation, <http://www.cris.com/~ketchup/genedoc.shtml>). We have described molecular modelling of 2C7 previously,<sup>1</sup> and the epitope

for monoclonal antibody 2G10 was viewed using the Swiss Pdb viewer.<sup>19 20</sup>

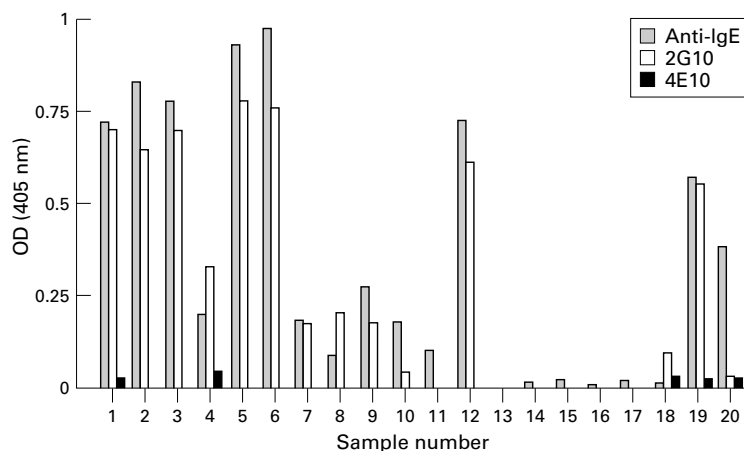
## Results

**REACTIVITY OF MONOCLONAL ANTIBODY 2G10 WITH MOUSE AND HUMAN IMMUNOGLOBULINS**  
We have generated two mouse monoclonal anti-idiotypes (2G10/IgG1 $\kappa$  and 4E10/IgG1 $\kappa$ ) directed against monoclonal antibody 2C7, as surrogate human IgE anti-Der p 1. Both anti-idiotypes were found to be specific for monoclonal antibody 2C7 because they did not bind to a panel of 20 other mouse monoclonal antibodies, including two isotype controls (IgG2b), two other anti-Der p 1 monoclonal antibodies (5H8 and 4C1), and 16 antibodies of irrelevant specificities (fig 1). However, monoclonal antibodies 2G10 and 4E10 did not block the binding of monoclonal antibody 2C7 or human IgE to Der p 1 (data not shown), thereby suggesting that they are directed against V region determinants not directly involved in Der p 1 binding.

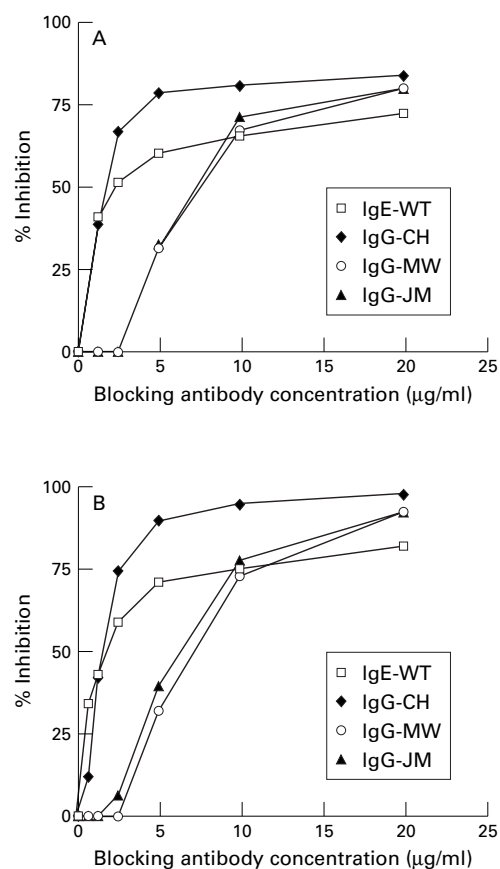
Interestingly, monoclonal antibody 2G10, but not monoclonal antibody 4E10, recognised



**Figure 1** Anti-idiotype monoclonal antibodies 2G10 and 4E10 are both specific for monoclonal antibody 2C7 (antibody number 1) because they do not bind to a panel of 20 other mouse monoclonal antibodies, including two isotype controls (IgG2b; antibody numbers 2 and 3), two other anti-Der p 1 monoclonal antibodies (5H8 and 4C1; antibody numbers 4 and 5),<sup>5</sup> and 16 antibodies of irrelevant specificities (antibody numbers 6–21).



**Figure 2** Reactivity of anti-idiotype monoclonal antibodies 2G10 and 4E10 with Fc $\epsilon$ RI bound IgE. Samples 1–6 are sera from atopic patients with IgE anti-Der p 1 (total IgE > 1000 IU/ml), 7–12 are sera from atopic patients with no IgE anti-Der p 1 (total IgE, 174 to > 1000 IU/ml), 13–18 are sera from non-atopic controls (total IgE < 100 IU/ml), 19 is an IgE $\lambda$  myeloma protein (IgE-WT), and 20 is a chimaeric mouse–human IgE anti-NP<sup>7</sup>



**Figure 3** Inhibition of monoclonal antibody 2G10 binding to (A) Fc $\epsilon$ RI-bound IgE-WT and (B) Fc $\epsilon$ RI-bound serum IgE by human myeloma proteins (IgE-WT and IgG-CH) and polyclonal human IgG (IgG-MW and IgG-JM).

Fc $\epsilon$ RI bound human IgE, irrespective of its antigenic specificity (fig 2). However, monoclonal antibody 2G10 is directed against the Fv (fragment variable) region of IgE, because it does not bind to a chimaeric mouse–human IgE anti-NP, consisting of mouse variable region domains and human IgE constant region domains.<sup>7</sup> We then tested the ability of human IgE and IgG to inhibit the binding of monoclonal antibody 2G10 to Fc $\epsilon$ RI bound human IgE. Both myeloma proteins, IgE-WT and IgG-CH, were found to be equally inhibitory, regardless of whether a myeloma IgE (IgE-WT) or a polyclonal serum IgE was used to coat the cells. However, approximately four-fold more polyclonal serum IgG (IgG-MW and IgG-JM) was required to achieve 50% inhibition of binding compared with the myeloma proteins (fig 3).

These data indicate that monoclonal antibody 2G10 recognises an idiotope that is present both on the IgE and IgG myeloma proteins, but is only present on approximately 25% of polyclonal serum IgG. This leads us to speculate that the idiotope recognised by monoclonal antibody 2G10 is encoded by a commonly used human V<sub>H</sub> gene family, most likely V<sub>H</sub>3, because members of this family have been shown previously to share more than 70% homology with the idiotope monoclonal antibody 2C7.<sup>1</sup> To investigate this further, we tested three human antibody preparations,

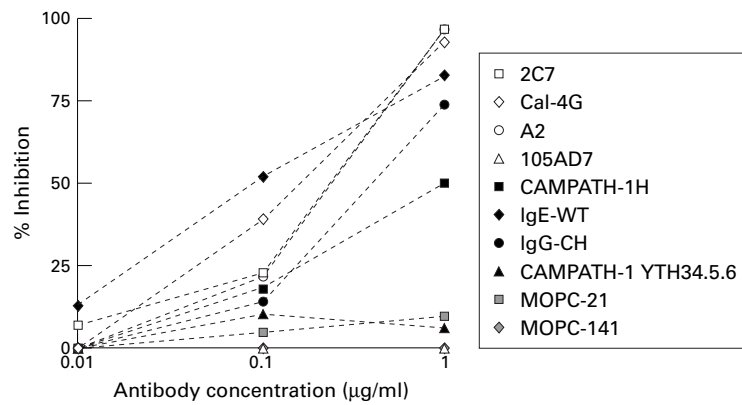


Figure 4 Inhibition of monoclonal antibody 2G10 binding to solid phase bound monoclonal antibody 2C7 by monoclonal antibody 2C7 and by three human monoclonal antibodies (Cal-4G, A2, and 105AD7), humanised CAMPATH-1H (complementarity determining region (CDR) grafted) antibody, two human myeloma proteins (IgE-WT and IgG-CH), rat CAMPATH-1 YTH34.5.6 antibody, and two mouse monoclonal antibodies (MOPC-21 and MOPC-141).

representative of different  $V_H$  gene families ( $V_H2$ ,  $V_H3$ , and  $V_H4$ ), for their ability to inhibit the binding of monoclonal antibody 2G10 to its idiotype monoclonal antibody 2C7 (fig 4). Both A2 ( $V_H3$ ) and Cal-4G ( $V_H4$ ) immunoglobulins inhibited the binding of monoclonal antibody 2G10 to the same extent as monoclonal antibody 2C7. However, monoclonal antibody 105AD7 ( $V_H2$ ) caused no such inhibition of binding, even at the highest concentration tested. Interestingly, the humanised CAMPATH-1H (CDR grafted) antibody, but not the original rat CAMPATH-1 YTH34.5.6 antibody, inhibited by about 50% the binding of monoclonal antibody 2G10 to monoclonal antibody 2C7, thereby indicating that monoclonal antibody 2G10 is directed against a framework region rather than the CDRs. Neither mouse monoclonal antibody MOPC-21,<sup>14 15</sup> nor mouse monoclonal antibody MOPC-141,<sup>16 17</sup> for which V region sequences are available, was inhibitory.

## A

```

2C7      : DVKVVESGGGLVQPGGSLKLSCTASGFDF. . SRYWMSVWRQAPGKLEWIGEINP. . DGSPINYP TSLKDKFII SRDN
CAL-4G   : Q-QLQW-A--LK-SET-S-T-AVY-GS. . -G-YW--I--P-----H. . S--.T--N---SRVT--V-T
A2       : Q-QM-----V---R-R--A---T-. . S-G-H--K-R--Q-----V-SY. . ---NKY-AD-V-GR-T---
CAMPATH-1H : Q-QLQ---P---R-SQT-S-T--V---T-. . TDFY-N---P--R-----F-RDKAK-YTTE-N--V-GRVTMLV-T
105AD7   : Q-TLK---PT--K-TQT-T-T--L---SLNT-GVCGV-I--P---A---LAH-YW. . DDK.R-S---SRLT-TK-T
MOPC-21  : --QL-----R---A---T-. . SFG-H-----E-----VAY-SS. . GS-TLH-ADTV-GR-T---
MOPC-141 : Q-QLEQ--P---A-SQ--SIT--V---SL. . TG-GVN---P-----L-T-W. . .GNGSTD-NST--SRLT-TK--
YTH34.5.6 : E--LL-----MR---AG--T-. . TDFY-N-I--PA--AP--L-F-RDKAK-YTTE-N--V-GR-T-----

```

SKNTLY	LQ
---QFS	-K
-----	--
---QFS	-R
---QVV	-T
P----F	--
--SQVF	-K
TQ-M--	--

```

2C7      : ISKVRSEDTALYYCARPGRLLHFDY      WGQCTTLTVSS
CAL-4G   : L-S-TAA--V---RIMYCSGGSCYSAGLRY   ----NLV---
A2       : MNSL-A---V---KWGGYCTNGVCYRGGYGMV --K-S-V---
CAMPATH-1H : L-S-TAA--V---EGHTAAPFDY          ----SLV---
105AD7   : MTNMDPV---T---QVLYDFWSGYLEYFAY   ----LV---
MOPC-21  : MTSL-----M---WGNYPYAMDY         ----SV---
MOPC-141 : MNSLQTD---R---SVSIYYGRSDKYFTLDY   ----SV---
YTH34.5.6 : MNTL-A---T---EGHTAAPFDY          ----VMV---

```

## B

```

2C7      : DVLMTQTPLSLPVSFQDQVSI SCRSSQSLANSYGNTYLSWYLHKPGQSPQLLIYGISDRFSGVDPDRFSGSGSDTFLKISTIKP
CAL-4G   : QPVL--P-S.ASA-L-AS-TLT-TL-SG. . . . -S-YKVD--QQR--KG-RFVVRVGTGGIV#I-----VL---LNRV-T-KN-QE
A2       : -IQ-----S--SA-L--R-T---A-----DI-N--N--RQ--DGTVK---YT-RLH---S-----YS-T--NLEQ
CAMPATH-1H : -IQ---S-S--SA-V--R-T-T-KA-- . . . . NIDK--N--QQ--KA-K---NTNQLT---S-----FT--SLQ-
105AD7   : -IEI--S-S--SA-V--R-R-T--A-- . . . . DISSF-N--Q---KA-K---AA-ILQ---S-----T-TILQ-
MOPC-21  : NIV---S-K-MSM-V-ER-TLT-KA-E . . . . NVV--V---QQ--E--K-----A-N-YT-----T---A---T--SVQA
MOPC-141 : -IQ--E-TAA-AA-L--R-T---A-- . . . . DI-NF-N-
YTH34.5.6 : -IK---S-SF-SA-V--R-TLN-KA-- . . . . NIDK--N--QQ-L-E--K---NTNQLT-I-S-----T--SLQ-

```

```

2C7      : EDLGMYYCLQGTHQPWT      FGGGKLEIKR
CAL-4G   : --ESD-H-GADHGSGSNFV
A2       : --IST-F-QQGNALPRT
CAMPATH-1H : --IAT---LQHISRPT      --Q---V---
105AD7   : G-FAT---QQSYKT PPS      --Q---KTNE
MOPC-21  : ---AD-H-GQGYSYPY
MOPC-141 :
YTH34.5.6 : --VAT-F-LQHISRPT      --T-----L-

```

For alignment, the amino acids GSKGDG were replaced by a # at position 57.

Figure 5 Protein sequence alignments of (A) the heavy chain and (B) the light chain of the monoclonal antibody 2C7 V region with sequences of three antibodies that react with monoclonal antibody 2G10 (human Cal-4G, human A2, and humanised CAMPATH-1H) and sequences of four antibodies that do not react with monoclonal antibody 2G10 (human 105AD7, mouse MOPC-21, mouse MOPC-141, and rat CAMPATH-1 YTH34.5.6). Complementarity determining regions (CDRs) are indicated by solid lines above the sequences; dots indicate missing amino acids. Boxed residues represent the hexapeptide sequence in monoclonal antibody 2G10 reactive antibodies and the corresponding sequences in those that are not reactive.



Figure 6 A molecular model of the Fv region of monoclonal antibody 2C7<sup>1</sup> showing the heavy chain (blue) and the light chain (yellow). The hexapeptide sequence is shown in space filling representation, with polar residues coloured red and the non-polar residue coloured green.

#### IDENTIFICATION OF A POSSIBLE IDIOTOPE FOR MONOCLONAL ANTIBODY 2G10

The V<sub>H</sub> and V<sub>L</sub> sequences of the different antibodies that were used in the monoclonal antibody 2G10 inhibition experiments were compared with the V<sub>H</sub> and V<sub>L</sub> sequences of monoclonal antibody 2C7 to identify the idiotope recognised by monoclonal antibody 2G10 (fig 5). Sequence analysis of the V<sub>H</sub> regions revealed the presence of a hexapeptide spanning residues 74–79 within framework region 3 (FRW3) of monoclonal antibody 2G10 reactive antibodies, which might comprise part of the epitope targeted by monoclonal antibody 2G10 (fig 6). This hexapeptide consists of polar amino acids in positions 74, 76, 77, and 79 of antibodies reactive with monoclonal antibody 2G10, whereas this pattern of amino acid distribution is not seen in antibodies that do not react with monoclonal antibody 2G10 (table 1). Given that both κ and λ light chains were found in the monoclonal antibody 2G10 reactive antibodies, we consider that V<sub>L</sub> does not make a major contribution to the reactivity with this anti-idiotypic.

#### Discussion

Our data suggest that monoclonal antibody 2G10 is directed against a crossreactive idiotope on human IgE that is shared by polyclonal

IgG, which is in keeping with an early report showing that antibodies raised against a single human myeloma IgE protein detected cross-reactive idiotope determinants on polyclonal IgG.<sup>21</sup> We have shown previously that monoclonal antibody 2C7, the idiotope against which monoclonal antibody 2G10 was raised, uses a germline gene from the mouse V<sub>H</sub>4 (X-24) family,<sup>1</sup> which is known to have considerable homology with the human V<sub>H</sub>3 gene family.<sup>22</sup> This leads us to speculate that the idiotope recognised by monoclonal antibody 2G10 is encoded by a commonly used human V<sub>H</sub> gene family, most likely V<sub>H</sub>3. Competitive inhibition studies against human immunoglobulins representative of V<sub>H</sub>2, V<sub>H</sub>3, and V<sub>H</sub>4 gene families confirmed that monoclonal antibody 2G10 is mostly likely to be directed against sequences encoded either by V<sub>H</sub>3 or V<sub>H</sub>4 genes.

It has been suggested that highly conserved FRW1 and FRW3 regions of murine and human antibodies encoding the V<sub>H</sub>3 gene segment might be responsible for their broad specificity.<sup>23</sup> Interestingly, binding to staphylococcal protein A is reported to be restricted to antibodies that use this gene segment, and non-binders are thought to have two or more amino acid differences at positions 74–77 in FRW3.<sup>24</sup> Given that the V<sub>H</sub> region of monoclonal antibody 2C7 shows up to 70% homology with the human V<sub>H</sub>3 gene family,<sup>1</sup> it is tempting to suggest that the idiotope for monoclonal antibody 2G10 is present on a conservative region of FRW3. Analysis of amino acids in the V<sub>H</sub> region for charge, hydrophobicity, and accessibility suggests that reactivity with monoclonal antibody 2G10 is defined by a hexapeptide spanning residues 74–79 within FRW3, which implies that the CDRs are not part of the idiotope. The fact that monoclonal antibody 2G10 binds to the humanised CAMPATH-1H (CDR grafted) antibody,<sup>9</sup> but not to the original rat CAMPATH-1 YTH34.5.6 antibody, is further evidence that it is directed against a FRW region, rather than the CDRs. This is, of course, very much in keeping with our observation that monoclonal antibody 2G10 does not block the binding of monoclonal antibody 2C7 or human IgE to Der p 1.

Our monoclonal antibody 2G10 anti-idiotypic could potentially be used as a probe for determining the contribution of the V<sub>H</sub>3 and V<sub>H</sub>4 gene segments to antigenic specificity. Furthermore, given that monoclonal antibody

Table 1 Characteristics of the monoclonal antibodies (mAbs) used for testing the reactivity of mAb 2G10

Antibody	Species	Isotype	Residues 74–79	V <sub>H</sub> family	V <sub>L</sub> family	Reactivity with mAb 2G10	Reference
2C7	Mouse	IgG2b	<b>S</b> <b>K</b> <b>N</b> <b>T</b> <b>L</b> <b>Y</b>	4	κ2	+	1
Cal-4G	Human	IgM	<b>S</b> <b>K</b> <b>N</b> <b>Q</b> <b>F</b> <b>S</b>	4–34	λ9	+	10, 11
A2	Human	IgA	<b>S</b> <b>K</b> <b>N</b> <b>T</b> <b>L</b> <b>Y</b>	3	κ10	+	12, 13
CAMPATH-1H	Human	IgG1	<b>S</b> <b>K</b> <b>N</b> <b>Q</b> <b>F</b> <b>S</b>	4	κ1	+	9
105AD7	Human	IgG1	<b>S</b> <b>K</b> <b>N</b> <b>Q</b> <b>V</b> <b>V</b>	2	κ1	–	8
MOPC-21	Mouse	IgG1	<b>P</b> <b>K</b> <b>N</b> <b>T</b> <b>L</b> <b>F</b>	2	κ19	–	14, 15
MOPC-141	Mouse	IgG2b	<b>S</b> <b>K</b> <b>S</b> <b>Q</b> <b>V</b> <b>F</b>	1	κ10	–	16, 17
YTH34.5.6	Rat	IgG2a	<b>T</b> <b>Q</b> <b>N</b> <b>M</b> <b>L</b> <b>Y</b>	2	κ1	–	9

Polar amino acids are shown in bold.

The V<sub>H</sub> of mAb 2C7 is highly homologous to the human V<sub>H</sub>3 gene family.<sup>1</sup> Antibodies A2<sup>12</sup> and the CDR grafted CAMPATH-1H<sup>9</sup> are recombinant proteins.

2G10 does not block the binding of Der p 1 to monoclonal antibody 2C7, it would be of great interest to investigate its molecular disposition in relation to antigen in a tri-molecular complex.

This work was supported by the BBSRC (Grant T03598). PBF is funded by a University of Nottingham international office scholarship and by CAPES-Governo Federal do Brasil (Processo BEX 1176/98-6). The authors are grateful to Dr K Potter for the gift of the human immunoglobulin proteins.

- 1 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.
- 2 Cooke GP, Tomlinson IM. The human immunoglobulin V<sub>H</sub> repertoire. *Immunol Today* 1995;16:237-42.
- 3 Tomlinson IM, Walter G, Marks JD, et al. The repertoire of human germ line VH segments reveals about fifty groups of VH segments with different hypervariable loops. *J Mol Biol* 1992;227:776-98.
- 4 Cox JPL, Tomlinson IM, Winter G. A directory of human germ line Vκ segments reveals a strong bias in the usage. *Eur J Immunol* 1994;24:827-36.
- 5 Chapman MD, Heymann PW, Platts-Mills TAE. Epitope mapping of two major inhalant allergens, Der p I and Der f I from mites of the genus dermatophagoides. *J Immunol* 1987;139:1479-84.
- 6 Takemoto H, Nishimura S, Kosada Y, et al. Anti-human IgE monoclonal antibodies recognising epitopes related to the binding sites of high and low affinity IgE receptors. *Microbiol Immunol* 1994;38:63-71.
- 7 Neuberger MS, Williams GT, Mitchell EB, et al. A hapten-specific chimaeric IgE antibody with human physiological effector function. *Nature* 1985;314:268-70.
- 8 Austin EB, Robins RA, Durrant LG, et al. Human monoclonal anti-idiotypic antibody to the tumour-associated antibody 79IT/36. *Immunology* 1989;67:525-30.
- 9 Riechmann L, Clark M, Waldmann H, et al. Reshaping human antibodies for therapy. *Nature* 1988;332:323-7.
- 10 Spellerberg MB, Chapman CJ, Mackridge I, et al. Dual recognition of lipid A and DNA by human antibodies encoded by the V<sub>H</sub>4-21 gene: a possible link between infection and lupus. *Hum Antibodies Hybridomas* 1995;6:52-6.
- 11 Williams SC, Winter G. Cloning and sequencing of human immunoglobulin Vλ gene segments. *Eur J Immunol* 1993;23:1456-61.
- 12 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 V<sub>H</sub>3-encoded Igs. *J Immunol* 1996;157:2982-8.
- 13 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.
- 14 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.
- 15 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.
- 16 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.
- 17 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.
- 18 Maurer D, Fiebigler E, Reininger B, et al. Expression of functional high affinity immunoglobulin E receptors (FcεRI) on monocytes of atopic individuals. *J Exp Med* 1994;179:745-50.
- 19 Guex N, Peitsch MC. SWISS-MODEL and the Swiss-Pdb viewer: an environment for comparative protein modelling. *Electrophoresis* 1997;18:2714-23.
- 20 Peitsch MC. ProMod and Swiss-Model: internet-based tools for automated comparative protein modelling. *Biochem Soc Trans* 1996;24:274-9.
- 21 Spiegelberg HL, Plummer JM, Ruedi J, et al. Lack of pokeweed mitogen-induced IgE formation in vitro by human peripheral blood mononuclear cells: detection of cross-reacting idiotypic determinants on polyclonal Ig by antibodies to a single IgE myeloma protein. *J Immunol* 1983;131:3001-5.
- 22 Haino M, Hayashida H, Miyata T, et al. Comparison and evaluation of human immunoglobulin V<sub>H</sub> segments located in the 3' 0.8-megabase region. *J Biol Chem* 1994;269:2619-26.
- 23 Schroeder HW, Jr, Hillson JL, Perlmutter RM. Structure and evolution of mammalian VH families. *Int Immunol* 1990;2:41-50.
- 24 Sasano M, Burton DR, Silverman GJ. Molecular selection of human antibodies with an unconventional bacterial B cell antigen. *J Immunol* 1993;151:5822-39.