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-idiotype

P B Furtado, R Furmonaviciene, J McElveen, H F Sewell, F Shakib

Abstract

Background—Two mouse monoclonal antibodies (mAbs) have been described recently; namely, mAb 2C7 (IgG2bxs), which is directed against the major house dust mite allergen Der p 1, and mAb 2G10 (IgG1xs), which is an anti-idiotypic antibody raised against mAb 2C7. The anti-idiotype 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–idiotype–anti-idiotype. 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-idiotype (mAb 2G10).

Keywords: Der p 1; anti-idiotype antibodies; monoclonal antibodies; antigen–antibody binding

We have recently described two mouse monoclonal antibodies; namely, mAb 2C7 (IgG2bxs), which is directed against the major house dust mite allergen Der p 1, and mAb 2G10 (IgG1xs), 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, and that this specificity is a major component of the human IgE anti-Der p 1 response. 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. Anti-idiotype mAb 2G10 appears to be directed against FRW sequences encoded by the V$_\gamma$3 and V$_\gamma$4 gene families, but its most intriguing property is that it reacts with human IgE regardless of its antigenic specificity. The fact that mAb 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, is further evidence that it is directed against a FRW region, rather than the CDRs. 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. This means that mAb 2C7 can simultaneously bind to Der p 1 and to mAb 2G10, thereby generating a trimolecular complex consisting of antigen–idiotype–anti-idiotype. 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 idiotype–anti-idiotype complexes in the literature. Two of these are based on the anti-idiotype mimicking the antigen and therefore reacting with the CDRs rather than the FRW regions, and the other two anti-idiotypes recognise CDR and FRW residues. 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 and Swiss PDB-Viewer, we have investigated shape and charge complementarity to predict the interacting surfaces in the trimolecular complex.
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, provide a rare insight into how an antigen might bind to its antibody while in complex with an anti-idiotype.

Materials and methods
PRODUCTION OF MOUSE MONOCLONAL ANTI-IDIOYPE 2G10
The anti-idiotype mAb 2G10 (IgG1) was produced and characterised as documented previously.2

REVERSE TRANSCRIPTION AND POLYMERASE CHAIN REACTION (PCR)
Approximately 1 × 10⁶ mAb 2G10 hybridoma cells were harvested by centrifugation and homogenised using a QIAшREDDER™ 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 µl of the total RNA preparation to 70°C for five minutes, then placing it on ice and adding 4 µl 5× Superscript buffer, 0.5 µl oligo(dT) primer (0.5 mg/ml; Pharmacia, Uppsala, Sweden), 2 µl dNTP (5 mM), 2 µl dithiothreitol (0.1 M), 0.6 µl RNasin™ ribonuclease inhibitor (40 U/ml; Promega Corporation, Madison, Wisconsin, USA), and 200 U Superscript II™ 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 µl 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 µg/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 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 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 PRISM™ 310 genetic analyser, using the ABI PRISM dye terminator cycle sequencing ready reaction kit (Perkin-Elmer Applied Biosystems, Warrington, UK).
Apart from the L1 region, the Kabat and Clothia systems agree in terms of amino acid numbering of the hypervariable loop regions. Numbers in bold represent residue positions according to the Kabat system.

Table 1 Canonical structure classification of the hypervariable loop regions of monoclonal antibody 2G10 V\textsubscript{H} and V\textsubscript{L} according to Kabat and co-workers\textsuperscript{13}, showing sequence similarities with known antibody structures (MCP60\textsuperscript{39}, H\textsubscript{Y}H\textsubscript{E}L\textsubscript{45}, and NC4\textsubscript{19}).

<table>
<thead>
<tr>
<th>Canonical structure</th>
<th>FRW residues</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>L1 region</strong></td>
<td></td>
</tr>
<tr>
<td>2C7</td>
<td>2G10</td>
</tr>
<tr>
<td>25* 26 27 28 29* 30 31 a b c d e f 28 29 30 31 32 33</td>
<td>I F</td>
</tr>
<tr>
<td>McP603 3</td>
<td></td>
</tr>
<tr>
<td>2S S E S L L N S G N E K N F L</td>
<td>I F</td>
</tr>
<tr>
<td><strong>L2 region</strong></td>
<td></td>
</tr>
<tr>
<td>2G10</td>
<td></td>
</tr>
<tr>
<td>50 51 52</td>
<td></td>
</tr>
<tr>
<td>McP603 1</td>
<td></td>
</tr>
<tr>
<td>G A S</td>
<td></td>
</tr>
<tr>
<td><strong>L3 region</strong></td>
<td></td>
</tr>
<tr>
<td>2G10</td>
<td></td>
</tr>
<tr>
<td>90* 91 92 93 94 95* 96*</td>
<td></td>
</tr>
<tr>
<td>McP603 1</td>
<td></td>
</tr>
<tr>
<td>N D H S Y P L</td>
<td></td>
</tr>
<tr>
<td><strong>H1 region</strong></td>
<td></td>
</tr>
<tr>
<td>2G10</td>
<td></td>
</tr>
<tr>
<td>31 32 34*</td>
<td>26* 27* 28 29* 30 31 94*</td>
</tr>
<tr>
<td>McP603 1</td>
<td></td>
</tr>
<tr>
<td>S H M</td>
<td></td>
</tr>
<tr>
<td><strong>H2 region</strong></td>
<td></td>
</tr>
<tr>
<td>2G10</td>
<td></td>
</tr>
<tr>
<td>52a b c 53 54 55*</td>
<td>71*</td>
</tr>
<tr>
<td>McP603 1</td>
<td></td>
</tr>
<tr>
<td>P – – Y N D S</td>
<td></td>
</tr>
<tr>
<td>HyHeL5 2</td>
<td></td>
</tr>
<tr>
<td>P – – G S G</td>
<td></td>
</tr>
<tr>
<td>NC41</td>
<td></td>
</tr>
<tr>
<td>T – – N T G</td>
<td></td>
</tr>
</tbody>
</table>

Apart from the L1 region, the Kabat\textsuperscript{13} and Clothia\textsuperscript{37} systems agree in terms of amino acid numbering of the hypervariable loop regions. Numbers in bold represent residue positions according to the Kabat system.\textsuperscript{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 DNASPLOT\textsuperscript{18}, which is available at the International ImMunoGeneTics database (http://imgt.cines.fr:8104).\textsuperscript{12} Sequence numbering was done according to the Kabat system.\textsuperscript{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 2G10\textsuperscript{2} 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).

**MOLECULAR MODELLING**

We have previously described molecular modelling of mAb 2C7.\textsuperscript{1} Molecular models of mAbs CAMPATH-IH\textsuperscript{42}, CAMPATH-IH YTH34.5.6\textsuperscript{4} (1BF0),\textsuperscript{44} and MOPC-21\textsuperscript{15} 16 (1IGC)\textsuperscript{17} were available from the protein database (PDB).\textsuperscript{18} Modelling of mAbs 105AD7,\textsuperscript{19} A2,\textsuperscript{20} 21 Cal-4G\textsuperscript{22} 23 and MOPC-141\textsuperscript{24} 25 were based on the following templates that are available from the PDB\textsuperscript{46}: 1FVD,\textsuperscript{46} 1HOU,\textsuperscript{47} 7FAB,\textsuperscript{48} and 1OAK.\textsuperscript{49} Modelling of mAb 2G10 V\textsubscript{H} and V\textsubscript{L} was based on 1MF2\textsuperscript{20} and 1AE6,\textsuperscript{50} respectively. All modellings were carried out using Swiss Model\textsuperscript{91} 0 and were viewed using Swiss PDB-Viewer.\textsuperscript{9} Molecular modelling of Der p 1 has been described previously,\textsuperscript{32} and the Der p 1 epitope (Leu147–Gln160) recognised by mAb 2C7 has been reported by us elsewhere.\textsuperscript{27}

**Results**

PCR PRODUCTS AND CLONING

PCR amplification of mAb 2G10 cDNA, using the light and heavy chain primers, in conjunction with the reverse \(\kappa\) and \(\gamma\) chain primers, resulted in a single band of approximately 650 bp with V\textsubscript{L}fr1b and V\textsubscript{H}fr1g, respectively. These products were cloned into the vector pCRT\textsuperscript{2} M.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 \(\kappa\) 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.\textsuperscript{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.\textsuperscript{1} The mAb 2G10 heavy and light chain V region sequences showed the greatest homology with members of the IG\textsubscript{H}V1 and IG\textsubscript{K}V3 murine gene families, respectively. According to the Kabat system,\textsuperscript{13} mAb 2G10 V\textsubscript{L} has an insertion of four residues, 27a to 27d, in the first hypervariable region (L-CDR\textsubscript{1}). Insertions of one residue, 52a, and three residues, 100a to 100c, occur in the second (H-CDR\textsubscript{2}) and third (H-CDR\textsubscript{3}) regions.

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

<table>
<thead>
<tr>
<th>Antibody</th>
<th>FRW1</th>
<th>FRW3</th>
<th>Reactivity with mAb 2G10</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2C7</td>
<td>19</td>
<td>74</td>
<td>81</td>
<td>82</td>
</tr>
<tr>
<td>Cal-4G</td>
<td>S S K N T L Y L Q + 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>R S K N T L Y L Q + 20, 21</td>
<td>22, 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAMPATH-IH</td>
<td>S S K N Q F S L R + 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>105AD7</td>
<td>S S K N V V L T - 19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOPC-21</td>
<td>K P K N T L F L Q - 15, 16</td>
<td>28, 29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOPC-141</td>
<td>S S K S Q V F L K - 24, 25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YTH34.5.6</td>
<td>R T Q N M L Y L Q - 4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.
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 VH. 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 Phe71, corresponding to the canonical structure 3. 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 (1MF2,32 1ACY,33 and 1GGB 34) and heavy chain (1AE6,35 1PLG,35 and 1FBI) (fig 2). 1MF2 (F11.2.32),32 1ACY (59.1),33 and
1GGB (50.1) 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. Because mAb 2G10 also has TyrL27d, modelling of its light chain was based on F11.2.32 VL, which also belongs to the murine VK3 subgroup and corresponds to the same canonical classification as mAb 2G10 L regions. Another template was chosen for modelling the VH 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. Thus, modelling of the mAb 2G10 VH region was based on 1AE6 (antitumour mAb CTM01), which shows sequence similarities and has the same loop length (H3 region inclusive) compared with the other homologous structures (1PLG and 1FB1) (fig 2). The models were built using Swiss Model and viewed using Swiss PDB-Viewer.

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) 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, Thr13H, and Lys 75H. The previously determined epitope for mAb 2C7 on Der p 1 is shown in purple.

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<sub>H</sub>3 and V<sub>H</sub>4 genes. It is known that codons 6–24 in FRW1 and 67–85 in FRW3 are highly conserved among sequences within a single V<sub>H</sub> family, but diverge when compared with different V<sub>H</sub> families. The variation of these sequences in FRW1 and FRW3 is considerably smaller when comparing mouse and human V<sub>H</sub>3 gene segments. 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<sub>H</sub>3 family, 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. Comparison of the three dimensional models of the mAb 2G10 reactive and non-reactive antibodies, using Swiss Model and Swiss PDB-Viewer, 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-4G2 and CAMPATH-1H, 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, MOPC-21, and MOPC-141, which were not reactive with mAb 2G10, 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-2112 has a non-polar residue in position 74, namely Pro, that might also influence its reactivity with mAb 2G10, because Ser in this position appears to be highly conserved in mAb 2G10 reactive antibodies from the VH3 and V14 gene families. In the original rat CAMPATH-1H YTH34.5.6 antibody, 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–mAb2 C7–mAb2 G10**

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. 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–Gln160) and mAb 2C7 CDRs using Swiss PDB-Viewer. Analysis of the molecular model of the complex formed between Der p 1 and mAb 2C7 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) 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 Der p 1 and mAb 2C7 surfaces in the mAb 2C7 CDRs. The electrostatic potential was computed by the program Swiss PDB-Viewer, which uses simple coulomb interaction.
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 (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 (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.

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Discussion
In this paper, we used the programs Swiss Model and Swiss PDB-Viewer 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), and which demonstrated that mAb 2G10 does not bind to the CDR regions of mAb 2C7. 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. Given that the anti-idiotype mAb 2G10 recognises FRW residues encoding human immunoglobulin V, 3 and V, 4 gene segments, it might be feasible to use this anti-idiotype in inhibition experiments to determine the influence of FRW regions of such antibodies on antigen binding. Furthermore, considering its broad IgE specificity, our anti-idiotype 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 FceRI bound IgE, and in doing so coligating FceRI with FcγRIb, which has been reported to be a negative regulator of type I allergic responses. 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-idiotype 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-idiotype (mAb 2G10).

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).


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