The production and characterisation of a chimaeric human IgE antibody, recognising the major mite allergen Der p 1, and its chimaeric human IgG1 anti-idiotype

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Background: Two mouse monoclonal antibodies have been described, namely: mAb 2C7 (IgG2b), which is directed against the major house dust mite allergen Der p 1, and mAb 2G10 (IgG1k), which is an anti-idiotypeic antibody raised against mAb 2C7. Given its broad IgE specificity, anti-idiotype mAb 2G10 could potentially have immunomodulatory applications. For example, a chimaeric human IgG version of mAb 2G10 could prove to be a useful molecule for binding to mast cell and basophil FcεRI bound IgE, and in doing so co-ligating FcγRII with FcγRIIB, which has been reported to have downregulatory effects.

Aims: To produce a chimaeric human IgE version of mAb 2C7 (mAb 2C7huE) and a chimaeric human IgG1 version of its anti-idiotype mAb 2G10 (mAb 2G10huG1).

Methods: The Vκ and VH regions of mAb 2C7 and its anti-idiotype mAb 2G10 were engineered into human constant regions of the IgE and IgG1 isotypes, respectively.

Results: The production of chimaeric mAb 2C7huE and its anti-idiotype mAb 2G10huG1 confirmed that the respective mouse antibody V regions were successfully engineered into human constant regions and still retained the specificity of the original murine V regions.

Conclusion: The newly constructed chimaeric antibodies will be useful to investigate the downregulation of IgE mediated hypersensitivity by the crosslinking of FcεRI with FcγRIIB.

ITAM is also found in antigen receptors, such as those of B and T cells, and IgG receptors, such as FcγRI and FcγRII. ITAM bearing receptors are negatively regulated by an immunoreceptor tyrosine based inhibition motif found on FcγRIIB. FcγRIIB, which is highly homologous in mice and humans, is a single chain low affinity receptor for IgG that is widely distributed on both lymphoid and myeloid cells. The negative regulatory effect of FcγRIIB is highlighted by the fact that FcγRIIB deficient mice show augmented FcεRI mediated mast cell activation. In keeping with these observations, the crosslinking of FcεRI with FcγRIIB has been shown to inhibit murine IgE induced bone marrow derived mast cell and basophilic leucocyte activation, in addition to B and T cell antigen receptor dependent activation. The crosslinking of FcεRI with FcγRIIB is thought to be a regulatory physiological event that could potentially be brought about by antigens that stimulate the production of both IgE and IgG antibodies.

We have recently described two mouse monoclonal antibodies (mAbs), namely: mAb 2C7 (IgG2b), which is directed against the major house dust mite allergen Der p 1, and mAb 2G10 (IgG1k), which is an anti-idiotypeic antibody raised against mAb 2C7. We have already established that anti-idiotype mAb 2G10 recognises framework (FRW) residues encoding human immunoglobulin VH3 and VH4 gene segments, but its most intriguing property is that it reacts with human IgE, regardless of its antigenic specificity. Therefore, given its broad IgE specificity, our anti-idiotype mAb 2G10 could potentially have immunomodulatory applications. For instance, a chimaeric human IgG version of mAb 2G10 could prove to be a useful molecule for binding to mast cell and basophil FcεRI bound IgE, and in doing so co-ligating FcεRI with FcγRIIB, which as indicated above has downregulatory effects.

In this article, we describe the production of a chimaeric human IgE version of mAb 2C7 (mAb 2C7huE) and a chimaeric human IgG1 version of its anti-idiotype mAb 2G10 (mAb 2G10huG1).

MATERIALS AND METHODS

Antibody reagents

Mouse anti-Der p 1 mAb 5H8was obtained from Indoor Biotechnologies Limited (Manchester, UK). A human myeloma

Abbreviations: CDR, complementarity determining region; ELISA, enzyme linked immunosorbent assay; FcεRI, high affinity IgE receptor; FcγRII, low affinity IgE receptor; FCS, fetal calf serum; FRW, framework region; Ig, immunoglobulin; IMDM, Iscove’s modified Dulbecco’s medium; ITAM, immunoreceptor tyrosine based activation motif; mAb, monoclonal antibody; PBS, phosphate buffered saline; PCR, polymerase chain reaction

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IgE (IgE-λ-WT) was purified by affinity chromatography from a plasma sample kindly provided by Professor D Stanworth (Peptide Therapeutics plc, Cambridge, UK). Mouse anti-Der p 1 mAb 2C7 (IgG2b κ) and its mouse anti-idiotype mAb 2G10 (IgG1 κ) were produced by conventional hybridoma technology. Both mAb 2C7 and mAb 2G10 were fully characterised before.

Overlapping extension PCR

Overlapping extension polymerase chain reactions (PCRs) were carried out using 0.5–1.0 µg/ml of plasmid DNA in a 50 µl reaction volume, containing 10mM dNTPs (Amersham Pharmacia Biotech, Uppsala, Sweden), 10 µl of 10x Pfu buffer (Invitrogen, San Diego, California, USA), and 1 U/µl Pfu polymerase (kindly provided by Dr P Tighe, University of Nottingham, Nottingham, UK). In each step, specific primers were used depending on which PCR was being performed, as described below. Amplification consisted of one cycle at 95°C for one minute, 25 cycles at 95°C for 45 seconds, 55°C for 45 seconds, and 72°C for one minute, then one cycle at 72°C for 10 minutes. The negative control consisted of the reaction mixture with no plasmid DNA. After PCR amplification, the products of each step were subjected to electrophoresis on a 1% agarose gel diluted in TBE buffer (Sigma, Poole, Dorset, UK), containing 0.2 µg/ml ethidium bromide (Sigma), and the DNA was visualised using an ultraviolet transilluminator. Molecular weight markers were a 100 bp DNA ladder (Promega Corporation, Madison, Wisconsin, USA) and a λDNA digested with HindIII (Kramel Biotech, Northumberland, UK). All PCR products were gel purified using a QIAquick gel extraction kit (Qiagen Limited, Dorking, UK).

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).

Production of chimaeric mouse–human anti-Der p 1 (mAb 2C7huE)

Reverse transcription, PCR, cloning, and sequencing of the mAb 2C7 Vκ and VH chains have been described previously.17 mAb 2C7 Vκ and VH chains were subcloned separately into the M13VHPCR1 vector,17 within a HindIII and BamHI site, by sequential overlapping extension PCR (as described above).
using specifically designed primers (MGW Biotech AG, Ebersberg, Germany). For the mAb 2C7 Vκ chain, the oligonucleotides consisted of 2C7VKB (5′-TCCACAGGTGTCCACTCCGATTTGTTGTGACCCAAAC-3′; coding strand) and 2C7VKF (5′-GAGGTTGTAAGGACTCACCTTTGATTTCCAGCTGGTCCCCCTCCGA-3′; complementary strand). For the mAb 2C7 VH chain, the oligonucleotides consisted of 2C7VHB (5′-TCCACAGGTGTCCACTCCCAGGTCCAGGTCAAGCTGCTCGAGTC-3′; coding strand) and 2C7VHF (5′-GAGGTTGTAAGGACTCACCTGAGGAGACGGTGACTG-3′; complementary strand).

Briefly, the procedure involving chimaerisation of mAb 2C7 consisted of exchanging the exons of the cloned mAb 2C7 Vκ and VH chains with those from the pSVHyg and pSVgpt vector systems, respectively. The HindIII–BamHI restriction digestion analysis of clones of monoclonal antibody (mAb) 2C7 V region ligated into human constant region containing vectors. Clones containing bands of the expected size for (A) mAb 2C7 Vκ (867 bp) and the pSVHyg hucκ vector (10 kb) (K8) and (B) mAb 2C7 VH (890 bp) and the pSVgpt hucγ vector (6.5 kb) (H1–H17) are shown. M1, 100 bp DNA ladder; M2, λDNA digested with HindIII; C, control (pSVgpt B2VH hucγ).

Production of chimaeric mouse–human anti-idiotypic
(mAb 2G10huG1)
Reverse transcription, PCR, cloning, and sequencing of the mAb 2G10 Vκ and VH chains have been described previously. The chimaerisation of mAb 2G10 was carried out as for mAb 2C7 (see above), using specifically designed primers (MGW Biotech AG). For the mAb 2G10 Vκ chain, the oligonucleotides consisted of 2G10VKB (5′-TCCACAGGTGTCCACT CCAGATTGTTGTGACCCAAAC-3′; coding strand) and 2G10VKF (5′-GAGGTTGTAAGGACTCACCTTTGATTTCCAGCTGGTCCCCCTCCGA-3′; complementary strand). For the mAb 2G10 VH chain, the oligonucleotides consisted of: 2G10VHB (5′-TCCACAGGTGTCCACTCCCAGGTCCAGGTCAAGCTGCTCGAGTC-3′; coding strand) and 2G10VHF (5′-GAGGTTGTAAGGACTCACCTGAGGAGACGGTGACTG-3′; complementary strand).

Briefly, the procedure involving the chimaerisation of mAb 2G10 also consisted of exchanging the exons of the cloned mAb 2G10 Vκ and VH chains with those from the pSVHyg and pSVgpt vector systems, respectively. Compared with that of mAb 2C7, this procedure involved an extra cloning step with the pCR 2.1-TOPO vector (Invitrogen). Ligation of the PCR products of the HindIII–BamHI fragments, containing mAb 2G10 VH and VH, was performed within the EcoRI site of the pCR 2.1-TOPO vector, using the TOPO cloning kit (Invitrogen).
Plasmids were purified using the QIAprep spin miniprep kit (Qiagen Limited) and bands containing mAb 2G10 Vκ and VH chains of the correct size were extracted using a QIAquick gel extraction kit (Qiagen Limited). HindIII–BamHI gel extracted fragments, containing mAb 2G10 Vκ and VH chains, were cloned into plasmids containing hucκ23 and hucγ1, of allotype G1m(1,17), by replacement of the V regions of the pSVhyg Fog 1 hucκ22 and pSVgpt B2VH huγ1, vectors, respectively. Ligations were performed using T4 DNA ligase (Roche Diagnostics Limited, Lewes, East Sussex, UK) overnight at 11°C, and the transformation of Top 10 competent cells (Invitrogen) was carried out by heat shock. Miniprep DNA derived from the colonies was screened for the presence of vector and insert by restriction enzyme digestion of plasmids, using HindIII and BamHI, and by sequencing clones.

**Transformation of YB2/0 mammalian cells**

Before transfection, plasmids containing chimaeric mAb 2C7 and mAb 2G10 Vκ and VH regions were purified, using an EndoFree plasmid maxi kit (Qiagen Limited). Aliquots of 20 µg of each chimaeric pSVhyg 2C7Vκ hucκ and pSVhyg 2G10Vκ hucκ and 10 µg of each chimaeric pSVgpt 2C7VH hucγ1 and pSVgpt 2G10VH hucγ1 vector were linearised with PvuI (Life Technologies Limited, Paisley, UK) and transfected into 7 × 10^6 YB2/0 cells. The transfected cells were plated out in 96 well flat bottom plates (Costar, High Wycombe, UK) in the presence of Iscove’s modified Dulbecco’s medium containing 2mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 5mM HEPES (Sigma), and 5% fetal calf serum (FCS) (IMDM complete). Transfectants were selected for resistance to 250 µg/ml xanthine (Sigma) and 0.8 µg/ml mycophenolic acid (Sigma).

**Screening for mAb 2C7huE and mAb 2G10huG1 production**

Eighteen days after transfection, culture supernatant of clones producing mAb 2C7huE and mAb 2G10huG1 were assayed by enzyme linked immunosorbent assay (ELISA) for the presence of IgE and IgG, respectively. A mouse monoclonal antihuman IgE antibody (clone GE1; Sigma), or goat antihuman IgG Fc-specific antibody (Sigma), and a horseradish peroxidase conjugated goat antihuman κ light chain antibody (Harlan Sera-lab, Loughborough, UK) were used as capture and
detection antibodies, respectively. Clones secreting mAb 2C7huE (2E3) and mAb 2G10huG1 (1A5 and 1D8) were allowed to grow to saturation for three weeks in IMDM complete, containing 2% FCS with antibiotic selection (250 µg/ml xanthine and 0.8 µg/ml mycophenolic acid). mAb 2C7huE was precipitated from the supernatant using a 50% saturated solution of ammonium sulfate and dialysed against phosphate buffered saline (PBS).

Assessment of the specificity of mAb 2C7huE

Briefly, anti-Derp1 mAb 5H8 (4 µg/ml) coated microtitre plates were used to capture Der p 1 (2 µg/ml) on to the solid phase. mAb 2C7huE was then added to the plates, followed by the addition of either goat antihuman IgE (κ chain specific) or goat antihuman IgG/A/M (γ, α and μ chain specific; Sigma) conjugated to alkaline phosphatase. To exclude the possibility that mAb 2C7huE was binding to the capture antibody, some mAb 5H8 coated plates were treated with PBS instead of Der p 1. A modification of this assay was used to confirm that chimaeric mAb 2C7huE contained both κ and ε chains. Microtitre plates (Nunc Maxisorp; Nunc, Paisley, UK) were coated with a 1/1000 dilution of mouse monoclonal anti-human IgE antibody (clone GE1; Sigma) and then purified mAb 2C7huE was added to the plate. Bound antibodies were detected using a 1/1000 dilution of alkaline phosphatase conjugated goat antihuman κ light chain antibody (Sigma).

Assessment of the specificity of mAb 2G10huG1

Briefly, microtitre plates were coated with murine mAb 2C7 (1 µg/ml) or goat antihuman IgG (1/50 000 dilution; Sigma). The cell culture supernatants of clones 1A5 and 1D8 of mAb 2G10huG1 (100 µl/well), which were allowed to grow to saturation for three weeks, were then added to the plate. Bound antibodies were detected using a 1/1000 dilution of...
alkaline phosphatase conjugated goat antihuman \( \kappa \) light chain antibody (Sigma). The negative control consisted of 0.05% (vol/vol) of PBS-Tween 20. Other controls consisted of 100 \( \mu l \) well of cell culture supernatant from clones 5G5 (mAb Fog1V \( \kappa \)/2G10VH-IgG1 \( \kappa \)) and 4A10 (mAb 2G10V \( \kappa \)/B2VH-IgG1 \( \kappa \)).

**RESULTS**

**Chimaerisation of the original mouse mAb 2C7 to mAb 2C7huE**

We have produced a human IgE version (mAb 2C7huE) of mouse mAb 2C7. mAb 2C7 V\( \kappa \) and VH chains were chimaerised to retain the original murine complementarity determining regions (CDRs) and FRW regions, but with the huc\( \kappa \)/CL and the huc\( \gamma \)/CH1 regions, respectively. The chimaerisation of mAb 2C7 V\( \kappa \) and VH chains consisted of adding the M13VHPCR1 vector extensions\(^{22}\) to the mAb 2C7 V regions. The complete DNA sequence of mAb 2C7 contains, at its 5\(^\prime\) end, the immunoglobulin (Ig) promoter, the eukaryotic exon and intron leader sequences, the last four amino acids of the leader region (GVHS) (which form part of the V region exon) and, at its 3\(^\prime\) end, the 5\(^\prime\) end of the first half of the V-C intron. The complete DNA sequence of the mAb 2C7 V\( \kappa \) and VH chains, containing the M13VHPCR1 extensions, was cloned into the PCR2.1-TOPO vector between two EcoRI sites (fig 5). The gel purified bands of the mAb 2C7 V\( \kappa \) and VH chains were redigested with BamHI and HindIII confirmed that the mAb 2C7 VH chain has been inserted into the huc\( \kappa \) constant region (fig 2). These results were also confirmed by sequencing the mAb 2C7 V\( \kappa \) and VH regions (data not shown).

The mAb 2C7 VH chain was then cloned into the pSV-VNP huc\( \kappa \) vector by replacing the mAb NPVH.\(^{32}\) This vector also provided another IgH enhancer sequence\(^{30}\) to the construct located upstream of the mAb 2C7 VH exons. The human IgE constant region (huc\( \gamma \)),\(^{31}\) derived from the plasmid vector pSVgpt NP huc\( \gamma \) was subcloned into the mAb 2C7 VH construct, which resulted in the chimaeric pSVgpt 2C7 VH IgE expression vector.

Analysis of the restriction digests with SalI and HindIII, BamHI and HindIII, SalI and BamHI, or just BamHI confirmed that the mAb 2C7 VH chain has been inserted into the huc\( \kappa \) constant region (fig 4). Similar restriction digests with BamHI and HindIII confirmed that the mAb 2C7 V\( \kappa \) chain has been inserted into the huc\( \kappa \) constant region (fig 2).

**Chimaerisation of the original mouse mAb 2G10 to mAb 2G10huG1**

We have produced a human IgG1 version (mAb 2G10huG1) of the mouse anti-idiotypic mAb 2G10. mAb 2G10 V\( \kappa \) and VH were chimaerised to retain the original murine CDRs and FRW regions, but with the huc\( \kappa \)/CL and the huc\( \gamma \)/CH1 regions, respectively. The chimaerisation of the mAb 2G10 V\( \kappa \) and VH chains consisted of adding the M13VHPCR1 vector extensions\(^{22}\) to the mAb 2G10 V regions.

The complete DNA sequence of mAb 2G10 contains, at its 5\(^\prime\) end, the Ig promoter, the eukaryotic exon and intron leader sequences, the last four amino acids of the leader region (GVHS) (which form part of the V region exon) and, at its 3\(^\prime\) end, the 5\(^\prime\) end of the first half of the V-C intron. The complete DNA sequence of the mAb 2G10 V\( \kappa \) and VH regions, containing the M13VHPCR1 extensions, was cloned into the PCR2.1-TOPO vector between two EcoRI sites (fig 5). The gel purified bands of the mAb 2G10 V\( \kappa \) and VH chains were redigested with BamHI and HindIII and cloned into plasmid vectors containing the huc\( \kappa \)–BamHI site into the pSV-VNP huc\( \kappa \) vector by replacing the mAb NPVH.\(^{32}\) This vector also provided another IgH enhancer sequence\(^{30}\) to the construct located upstream of the mAb 2G10 VH exons. The unique IgE constant region (huc\( \gamma \)),\(^{31}\) derived from the plasmid vector pSVgpt NP huc\( \gamma \) was subcloned into the mAb 2G10 VH construct, which resulted in the chimaeric pSVgpt 2G10 VH IgE expression vector.
and hucγ1 constant regions, respectively. Restriction 
digests with NotI and BamHI, NotI and HindIII, or HindIII 
and BamHI confirmed that the mAb 2G10 VH chain had 
been inserted into the constant hucγ1 region (fig 6).

Similar restriction digests with EcoRI and HindIII confirmed 
that the mAb 2C7 Vκ chain has been inserted into the 
constant hucκ region (fig 7). These results were also 
confirmed by sequencing the mAb 2G10 Vκ and VH regions 
(data not shown).

**Specificity of mAb 2C7huE**

Three weeks after transfection, the ELISA test showed that 
clone 2E3 was producing good quantities of mAb 2C7huE. In 
this test, the plates were coated with mAb 5H8, which 
recognises a different Der p 1 epitope to mAb 2C7, thereby 
exposing the epitope that is recognised by mAb 2C7. Thus, purified 
mAb 2C7huE recognised Der p 1, which was bound to mAb 
5H8, and was detectable by an antihuman Fcε specific 
antibody, but not with antihuman IgG/M/A. These data 
confirm the IgE identity and Der p 1 reactivity of mAb 2C7huE 
(fig 8). To verify that antibodies from clone 2E3 also contain 
the κ light chain, another ELISA test was carried out. Purified 
mAb 2C7huE antibodies from clone 2E3, which were captured 
with an antihuman IgE antibody, were detectable with an 
antihuman κ light chain antibody, thereby further confirming 
its IgEκ identity (fig 8).
Specificity of mAb 2G10huG1

mAb 2G10huG1 (clones 1A5 and 1D8) recognised the original mouse mAb 2C7 antibody and was detectable by an antihuman κ light chain antibody (fig 9). Furthermore, recognition of murine mAb 2C7 antibody was dependent on both mAb 2G10huG1 Vκ and VH chains, because antibodies with substitutions of mAb 2G10 Vκ for mAb Fog1Vκ (clone 5G5) and mAb 2G10 VH for mAb B2VH (clone 4A10) did not recognise mAb 2C7 (fig 9). Using antihuman IgG instead of murine mAb 2C7 as the capture antibody confirmed the isotype identity of mAb 2G10huG1. Replacing mAb 2G10huG1 with PBS-Tween showed that there was no crossreactivity between the capture antibody and the detecting antibody (fig 9).

DISCUSSION

The use of recombinant DNA techniques to construct antibody genes for producing novel chimaeric/humanised antibodies by mammalian cell transfection has been widely used to understand, and attempt to modulate, the effector functions of antibody molecules. The design and optimisation of degenerate primers that amplify conserved sequences of the Fd fragments (VH and CH1) and complete κ light chains of mouse monoclonal antibodies, combined with the use of specifically constructed vectors for subsequent cloning, have further facilitated the process of antibody chimaerisation. We have used these techniques to construct and produce two chimaeric antibodies, namely mAb 2C7huE and mAb...
2G10huG1, derived from mouse mAb 2C7 and mouse mAb 2G10, respectively. The newly constructed mAb 2C7huE consists of the original mouse Vk and VH domains combined with the human Ck, CH1, and Fce regions. The newly constructed mAb 2G10huG1 also retained the original mouse Vk and VH domains combined with the human Ck, CH1, and FcγI regions.

The production of chimaeric mAb 2C7huE and its anti-idiotypic mAb 2G10huG1 confirmed that the respective mouse antibody V regions were successfully engineered into human constant regions and still retained the specificity of the original murine V regions. mAb 2C7huE was shown to recognise Der p 1 specifically and also to contain human e and k constant regions. Chimaeric mAb 2C7huE was detectable by an antihuman IgE antibody, but not by a polyclonal antihuman IgG, IgM, or IgA antibody, thereby confirming its isotype identity. Chimaeric mAb 2G10huG1 was shown to recognise mAb 2C7 and also to contain human y and κ constant regions.

We expect monoclonal antibodies 2C7huE and 2G10huG1 to exhibit the effector functions of the appropriate human immunoglobulin constant regions; namely, binding to Fce and Fcγ receptors, respectively.

When chimaerising both antibodies, the first step was to design primers that would not only amplify the 3' and the 5' ends of the V regions, but also add extensions that overlap with the cloning vector M13VHPCR1.2 The M13VHPCR1 vector provided the Ig promoter, the eukaryotic exon and intron leader sequences, and the first half of the V-C intron. The second step involved sequential overlapping extension PCRs, inserting the cDNA of the original mouse V regions into the M13VHPCR1 vector within a HindIII–BamHI restriction site. This step was important to allow subsequent cloning of the mouse V regions into the pSV expression vectors containing the human constant regions (huc or huκ), the second half of the V-C intron, the SV40 polyadenylation site, the SV40 origin of replication, and selectable markers. Because the pSV based vectors were provided with Vk22–25 and VH22–25 regions derived from other monoclonal antibodies, the final step involved cloning our V regions by swapping the fragments within the HindIII–BamHI restriction site. This final step resulted in a complete chimaeric mouse/human Ig version of the original mouse monoclonal antibody, which was subsequently transfected into mammalian cells for expression.

“This take home messages

- We have produced a human–mouse chimaeric monoclonal antibody (mAb) directed against the major house dust mite allergen Der p 1 (2C7huE) and its anti-idiotypic mAb 2G10huG1
- We have confirmed that the respective mouse antibody V regions were successfully engineered into human constant regions and still retained the specificity of the original murine V regions
- The newly constructed chimaeric antibodies will be useful to investigate downregulation of IgE mediated hypersensitivity by the crosslinking of FceRI with FcγRIIB

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Figure 8  Dose dependent binding of monoclonal antibody (mAb) 2C7huE, from clone 2E3, to Der p 1, captured on to (A) mAb 5H8 or (B) antihuman IgE. Purified mAb 2C7huE was detectable with antihuman Fcε specific antibody (squares) and antihuman κ light chain antibody (triangles), but not with polyclonal antihuman Fcγ, Fca, or Fcγ specific antibodies (circles). OD, optical density.

Figure 9  Confirmation of the monoclonal antibody (mAb) 2C7 reactivity and IgG identity of mAb 2G10huG1. The antibodies in cell culture supernatants (100 µl/well) of positive clones 1A5 and 1D8 bound to immobilised mAb 2C7 (open bars) or to immobilised antihuman IgG (solid bars) and were detectable with antihuman Fcε specific antibody (squares) and antihuman κ light chain antibody (triangles), but not by a polyclonal antihuman Fcγ, Igκ, IgM, or IgA antibody, thereby confirming its isotype identity. Chimaeric mAb 2G10huG1 was shown to recognise Der p 1 (2C7huE) and its anti-idiotype mAb 2G10huG1 confirmed that the respective mouse antibody V regions were successfully engineered into human constant regions and still retained the specificity of the original murine V regions. mAb 2C7huE was shown to recognise Der p 1 specifically and also to contain human e and k constant regions. Chimaeric mAb 2C7huE was detectable by an antihuman IgE antibody, but not by a polyclonal antihuman IgG, IgM, or IgA antibody, thereby confirming its isotype identity. Chimaeric mAb 2G10huG1 was shown to recognise mAb 2C7 and also to contain human y and κ constant regions.

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REFERENCES