ORIGINAL ARTICLE

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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J Clin Pathol: Mol Pathol 2002;**55**:315–324

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Accepted for publication 21 June 2002

Background: Two mouse monoclonal antibodies have been described, namely: mAb 2C7 (IgG2bκ), which is directed against the major house dust mite allergen Der p 1, and mAb 2G10 (IgG1κ), which is an anti-idiotypic antibody raised against mAb 2C7. 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 FcεRI 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.

It is now well established that the development of atopic diseases, such as asthma, is linked to circulating concentrations of IgE, the antibody class responsible for allergic hypersensitivity reactions. IgE binds to mast cells and basophils via its high affinity receptor (FcεRI), and subsequent crosslinking of FcεRI bound IgE molecules by allergen leads to the release of the mediators responsible for allergic tissue damage.[1] FcεRI consists of four transmembrane polypeptides, namely: α, β, and two disulphide bonded γ chains. It is the α chain that binds IgE, whereas the β and the γ chains are required for signal transduction and membrane insertion, respectively.[2] Although the early signal transduction mechanism necessary for activating the β subunit of FcεRI is not yet fully understood, this subunit is known to contain a conserved immunoreceptor tyrosine based activation motif (ITAM) in its cytoplasmic tail.[3]

> "Given its broad IgE specificity, our anti-idiotype monoclonal antibody 2G10 could potentially have immunomodulatory applications"

ITAM is also found in antigen receptors, such as those of B and T cells,[4 5] and IgG receptors, such as FcγRI and FcγRIII.[6 7] ITAM bearing receptors are negatively regulated by an immunoreceptor tyrosine based inhibition motif found on FcγRIIB.[8] FcγRIIB, which is highly homologous in mice and humans,[9] is a single chain low affinity receptor for IgG[10] that is widely distributed on both lymphoid and myeloid cells.[11] The negative regulatory effect of FcγRIIB is highlighted by the fact that FcγRIIB deficient mice show augmented FcεRI mediated mast cell activation.[12 13] 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,[14] in addition to B and T cell antigen receptor dependent activation.[8 15] The crosslinking of FcεRI with FcγRIIB is thought to be a regulatory physiological event[16] that could potentially be brought about by antigens that stimulate the production of both IgE and IgG antibodies.[8]

We have recently described two mouse monoclonal antibodies (mAbs), namely: mAb 2C7 (IgG2bκ), which is directed against the major house dust mite allergen Der p 1,[17 18] and mAb 2G10 (IgG1κ), which is an anti-idiotypic antibody raised against mAb 2C7.[19 20] We have already established that anti-idiotype mAb 2G10 recognises framework (FRW) residues encoding human immunoglobulin VH3 and VH4 gene segments,[20] but its most intriguing property is that it reacts with human IgE regardless of its antigenic specificity.[19] 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 5H8[21] was 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; FcγRIIB, 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

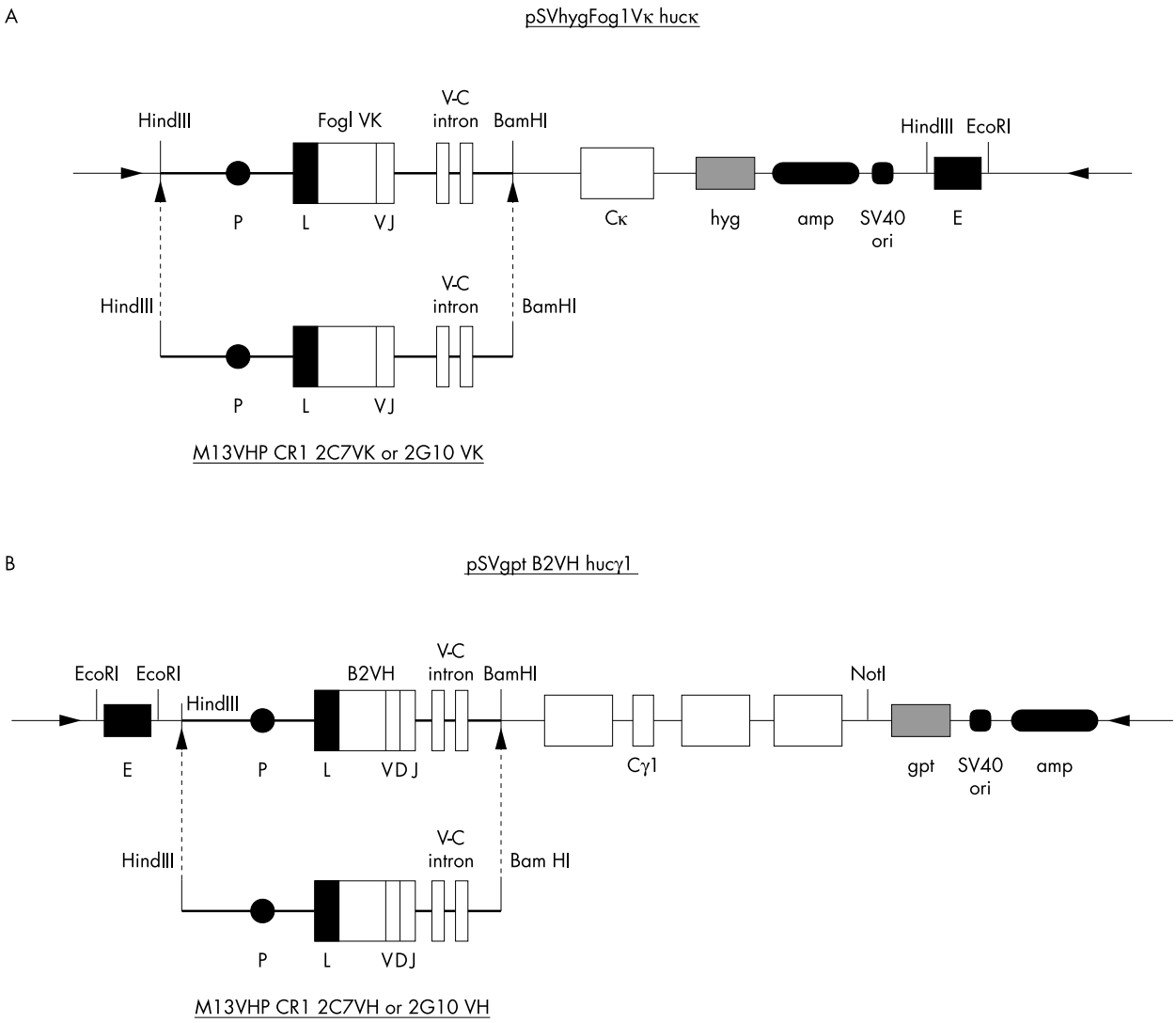

Figure 1 Schematic representation of the chimaerisation of monoclonal antibody (mAb) 2C7 and mAb 2G10 V regions into the hucκ and hucγ1 containing vectors. mAb 2C7 and mAb 2G10 Vκ (VJ) and VH (VDJ) chains were cloned into (A) hucκ and (B) hucγ1 containing vectors, respectively. amp, ampicillin; E, enhancer; gpt, xanthine-guanine phosphoribosyl transferase gene; hyg, hygromycin; L, leader; ori, origin; P, promoter.

IgE (IgEλ-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 (IgG2bκ)[17] and its mouse anti-idiotype mAb 2G10 (IgG1κ)[19] were produced by conventional hybridoma technology. Both mAb 2C7[17 18] and mAb 2G10[19 20] 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 10× 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,[22] within a HindIII and BamHI site, by sequential overlapping extension PCR (as described above)

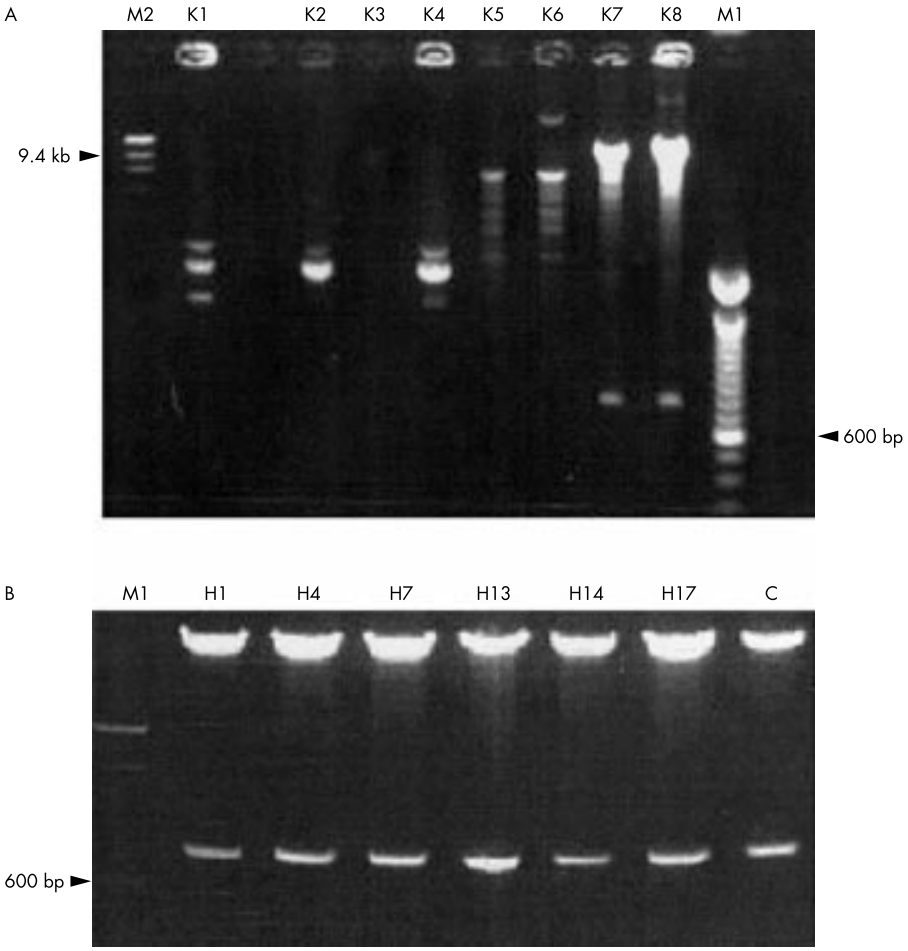

Figure 2 HindIII and BamHI restriction digestion analysis of clones of monoclonal antibody (mAb) 2C7 V region ligated into human constant region containing vectors. Clones containing bands of the expected size for (A) mAb 2C7 Vκ (867 bp) and the pSVhyg hucκ vector (10 kb) (K8) and (B) mAb 2C7 VH (890 bp) and the pSVgpt hucγ1 vector (6.5 kb) (H1–H17) are shown. M1, 100 bp DNA ladder; M2, λDNA digested with HindIII; C, control (pSVgpt B2VH hucγ1).

using specifically designed primers (MGW Biotech AG, Ebersberg, Germany). For the mAb 2C7 Vκ chain, the oligonucleotides consisted of 2C7VKB (5′-TCCACAGGTGTCCACT CCGATGTTGTGGTGACCCAAAC-3′; coding strand) and 2C7VKF (5′-GAGGTTGTAAGGACTCACCTTTGATTTCCAGCT TGG-3′; complementary strand). For the mAb 2C7 VH chain, the oligonucleotides consisted of 2C7VHB (5′-TCCACA GGTGTCCACTCCGAGGTCAAGCTGCTCGAGTC-3′; coding strand) and 2C7VHF (5′-GAGGTTGTAAGGACTCACC TGAGGAGACTGTGAGAG-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 fragment containing the mAb 2C7 Vκ and VH chains was cloned into a human constant Igκ (hucκ)[23] and a human constant IgG1 (hucγ1), of allotype G1m (1,17),[24] by replacement of the V regions of pSVhyg Fog 1 hucκ[22 25 26] and pSVgpt B2 hucγ1,[27 28] vectors, respectively. mAb 2C7 VH was further subcloned into the pSVgpt human 4 vector, derived from pSVgpt CAMPATH Hu4VH vector,[27 29] to add an IgH enhancer sequence to the construct.[30] The chimaeric pSVgpt 2C7 VH hucε expression vector was constructed by extraction and cloning of the BamHI fragment containing the hucε region[31] from the pSV-VNP hucε vector.[32] Ligations were performed using T4 DNA ligase "ready to go" (Amersham Pharmacia Biotech) and the HB101 cells (Promega Corporation) were transformed by electroporation. Colonies were screened for the presence of vector and insert by restriction enzyme digestion of plasmids, using HindIII and BamHI, and by sequencing clones.

Production of chimaeric mouse–human anti-idiotype (mAb 2G10huG1)

Reverse transcription, PCR, cloning, and sequencing of the mAb 2G10 Vκ and VH chains have been described previously.[20] 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′-TCCACAGG TGTCCACTCCAACATTGTGCTGACCCAGTT-3′; coding strand) and 2G10VKF (5′-GAGGTTGTAAGGACTCACCTTTTATTTCC AGCCTGGTCCCCCCTCCGA-3′; complementary strand). For the mAb 2G10 VH chain, the oligonucleotides consisted of: 2G10VHB2 (5′-TCCACAGGTGTCCACTCCCAGGTCCAGTTGCA GCAGTCTGGACC-3′; coding strand) and 2G10VHF (5′-GAG GTTGTAAGGACTCACCTGAGGAGACGGTGACTG-3′; complementary strand).

Briefly, the procedure involving the chimaerisation of mAb 2G10 also consisted of exchanging the exons of 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 Vκ and VH, was performed within the EcoRI site of the pCR 2.1-TOPO vector, using the TOPO cloning kit (Invitrogen).

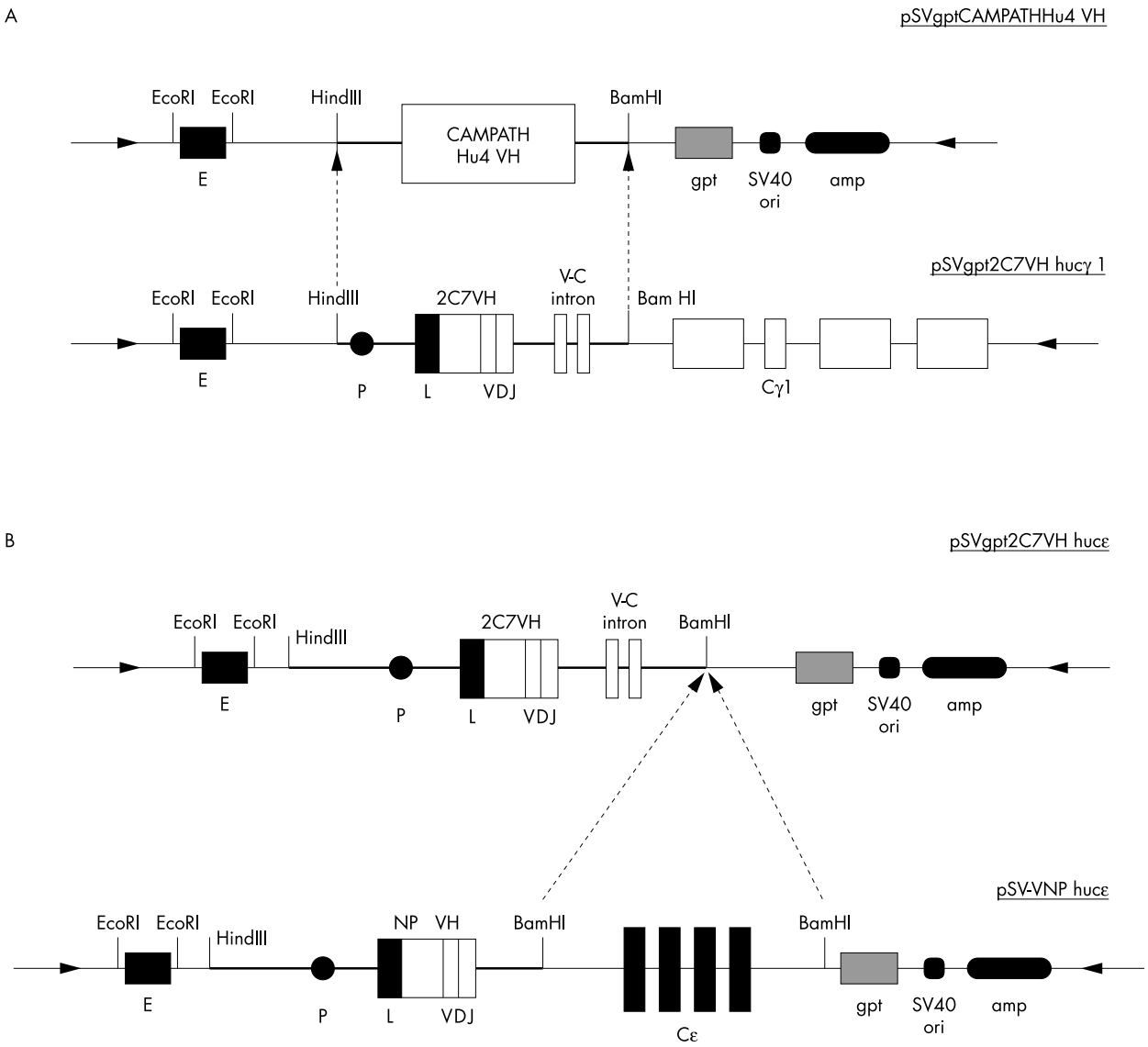

Figure 3 Schematic representation of the production of a chimaeric IgE version of monoclonal antibody (mAb) 2C7 VH. (A) The mAb 2C7 VH chain was cloned into a pSVgpt vector, encoded within a HindIII–BamHI site containing the IgH enhancer (E) (pSVgpt 2C7VH), followed by (B) the full chimaerisation of the mAb 2C7 VH chain to a human IgE version (pSVgpt 2C7VH hucε). amp, ampicillin; gpt, xanthine-guanine phosphoribosyl transferase gene; L, leader; ori, origin; P, promoter.

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 hucκ[23] and hucγ1, of allotype G1m(1,17),[24] by replacement of the V regions of the pSVhyg Fog 1 hucκ[22 25 26] and pSVgpt B2VH huγ1,[27 28] 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 V 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.[33] 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

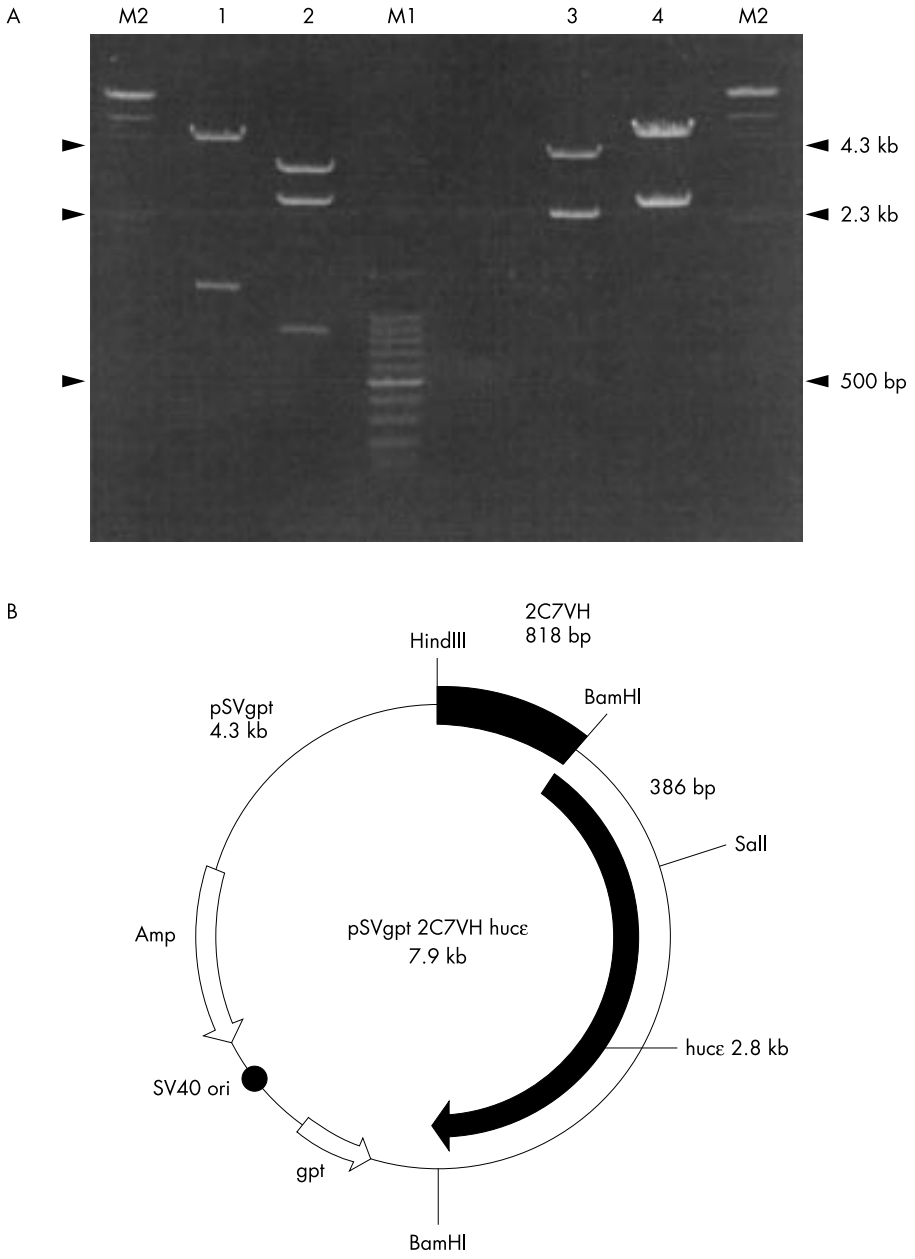

Figure 4 (A) Restriction digestions with (1) SalI and HindIII (6.8 kb and 1.2 kb), (2) BamHI and HindIII (4.3 kb, 2.8 kb, and 818 bp), (3) SalI and BamHI (5.1 kb, 2.5 kb, and 386 bp), or (4) just BamHI (5.1 kb and 2.8 kb) were used to confirm that (B) the monoclonal antibody 2C7 VH chain had been inserted into the hucε region containing vector in the correct orientation. M1, 100 bp DNA ladder; M2, λDNA digested with HindIII. Amp, ampicillin; gpt, xanthine-guanine phosphoribosyl transferase gene; ori, origin.

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-Der p 1 mAb 5H8 (4 µg/ml)[21] 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 (ϵ 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 plates. Bound antibodies were detected using a 1/1000 dilution of

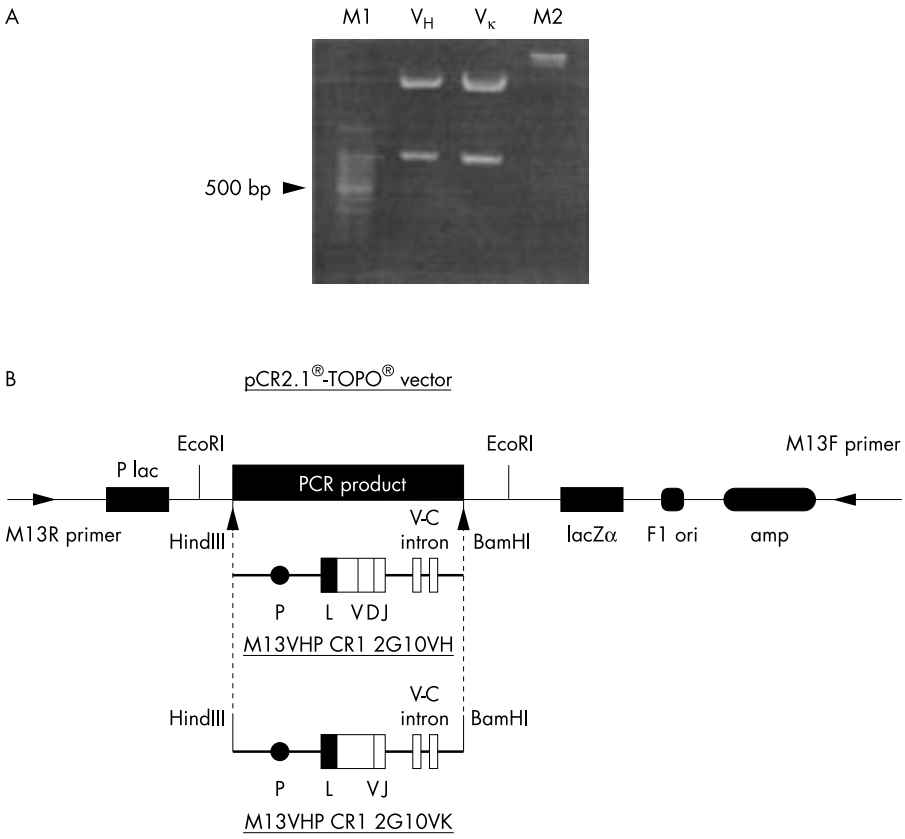

Figure 5 (A) Restriction digestion with EcoRI of clones containing monoclonal antibody 2G10 Vκ and VH chains (B) inserted into the pCR 2.1-TOPO vector. M1, 100 bp DNA ladder; M2, λDNA digested with HindIII. amp, ampicillin; L, leader; ori, origin; P, promoter.

alkaline phosphatase conjugated goat antihuman κ light chain antibody (Sigma). The negative control consisted of 0.05% (vol/vol) of PBS-Tween 20. Other controls consisted of 100 µl/well of cell culture supernatant from clones 5G5 (mAb Fog1Vκ/2G10VH- IgG1κ) and 4A10 (mAb 2G10Vκ/B2VH-IgG1κ).

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 Vκ and VH chains were chimaerised to retain the original murine complementarity determining regions (CDRs) and FRW regions, but with the hucκ/CL and the hucε/CH1 regions, respectively. The chimaerisation of mAb 2C7Vκ 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′ 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′ end, the 5′ end of the first half of the V-C intron. The complete DNA sequences of mAb 2C7 Vκ and VH chains, with the M13VHPCR1 extensions, were therefore cloned into plasmid vectors containing the hucκ and hucγ1 constant regions, respectively (fig 1). Clones containing the pSVhyg hucκ (10 kb) and mAb 2C7 Vκ (867 bp) or pSVgpt hucγ1 (6.5 kb) and mAb 2C7 VH (890 bp) were analysed by restriction digestion with BamHI and HindIII (fig 2).

The mAb 2C7 VH chain was then cloned into the pSVgpt human 4 vector, derived from the pSVgpt CAMPATH Hu4VH vector,[27 29] which does not have a constant region (fig 3). This stage was necessary because the pSV-VNP hucε vector,[32 34] containing the Fcε region,[31] had more than just one HindIII and BamHI restriction site. This would have impaired the direct cloning of the mAb 2C7 VH construct (within a HindIII–BamHI site) into the pSV-VNP hucε 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 (hucε),[31] derived from the plasmid vector pSVgpt NP hucε[32] (fig 3) was subcloned into the mAb 2C7 VH construct, which resulted in the chimaeric pSVgpt 2C7 VH hucε 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 hucε constant region (fig 4). Similar restriction digests with BamHI and HindIII confirmed that the mAb 2C7 Vκ chain has been inserted into the hucκ constant region (fig 2). These results were also confirmed by sequencing the mAb 2C7 Vκ and VH regions (data not shown).

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

We have produced a human IgG1 version (mAb 2G10huG1) of the mouse anti-idiotype mAb 2G10. mAb 2G10 Vκ and VH were chimaerised to retain the original murine CDRs and FRW regions, but with the hucκ/CL and the hucγ1/CH1 regions, respectively. The chimaerisation of the mAb 2G10 Vκ and VH chains consisted of adding the M13VHPCR1 vector extensions[22] to the mAb 2G10 V regions, using the same procedure described for mAb 2C7. The complete DNA sequence of mAb 2G10 contains, at its 5′ 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′ end, the 5′ end of the first half of the V-C intron. The complete DNA sequence of the mAb 2G10 Vκ 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 2G10 Vκ and VH chains were redigested with BamHI and HindIII and cloned into plasmid vectors containing the hucκ

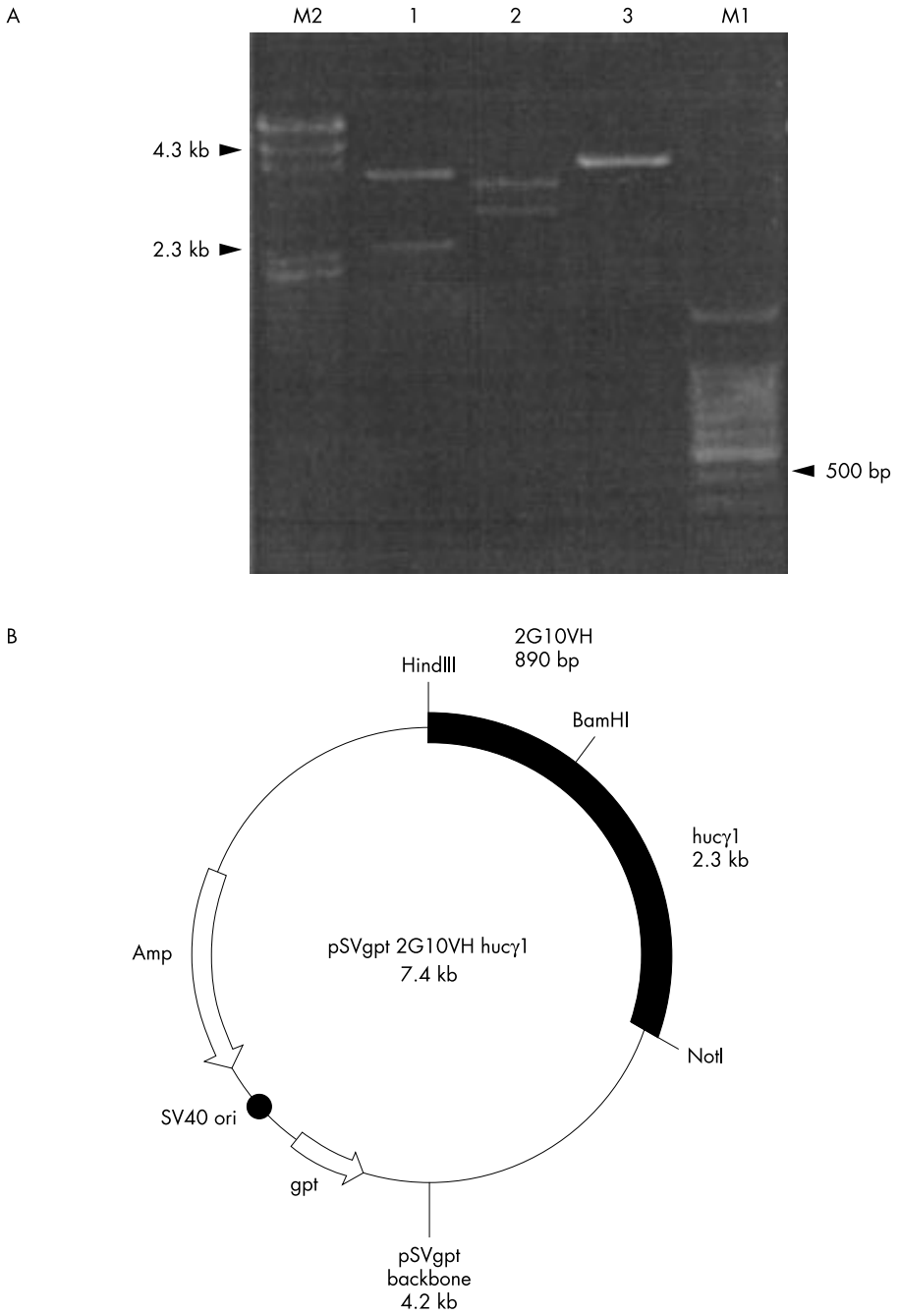

Figure 6 Restriction digestions of pSVgpt 2G10VH hucγ1 with (1) NotI and BamHI (5.0 kb and 2.3 kb), (2) NotI and HindIII (4.2 kb and 3.1 kb), and (3) HindIII and BamHI (6.5 kb and 890 bp) showing that (A) the monoclonal antibody 2G10 VH chain has been inserted into pSVgpt hucγ1 and (B) the localisation of the restriction sites on the pSVgpt 2G10VH hucγ1 vector. M1, 100 bp DNA ladder; M2, λDNA digested with HindIII. Amp, ampicillin; gpt, xanthine-guanine phosphoribosyl transferase gene; ori, origin.

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,[21] which recognises a different Der p 1 epitope to mAb 2C7,[18] 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).

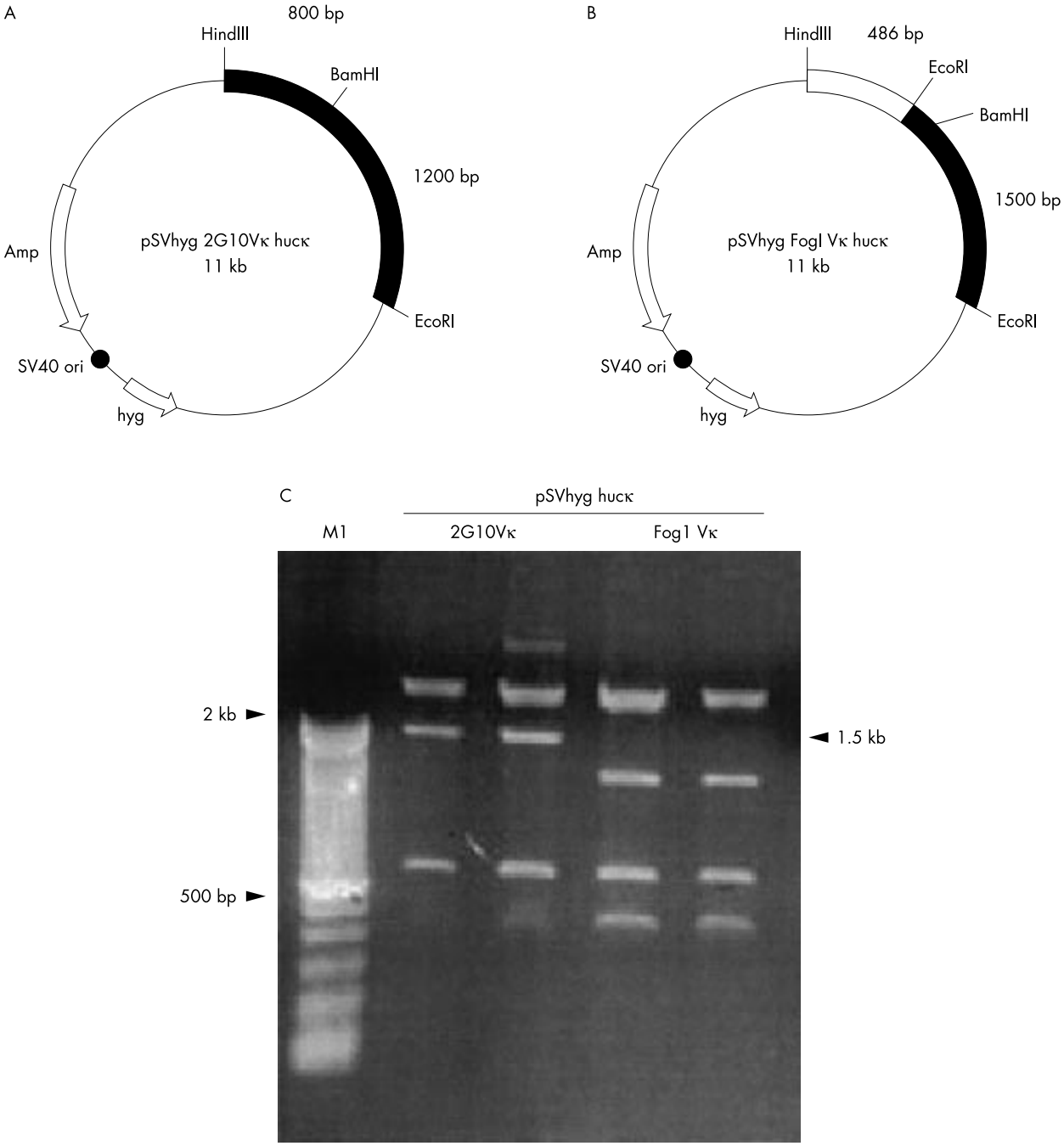

Figure 7 EcoRI and HindIII restriction digestion analysis of transformed clones with the pSVhyg 2G10Vκ hucκ vector. (A) The monoclonal antibody (mAb) 2G10 Vκ chain is not cut by EcoRI, whereas (B) mAb Fog1Vκ has one EcoRI site at position 468 downstream from the HindIII site. (C) The other released fragments correspond to other EcoRI sites present in the pSVhyg hucκ vector. M1, 100 bp DNA ladder.

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.[35–37] 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,[22 38] combined with the use of specifically constructed vectors for subsequent cloning,[22 39] 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

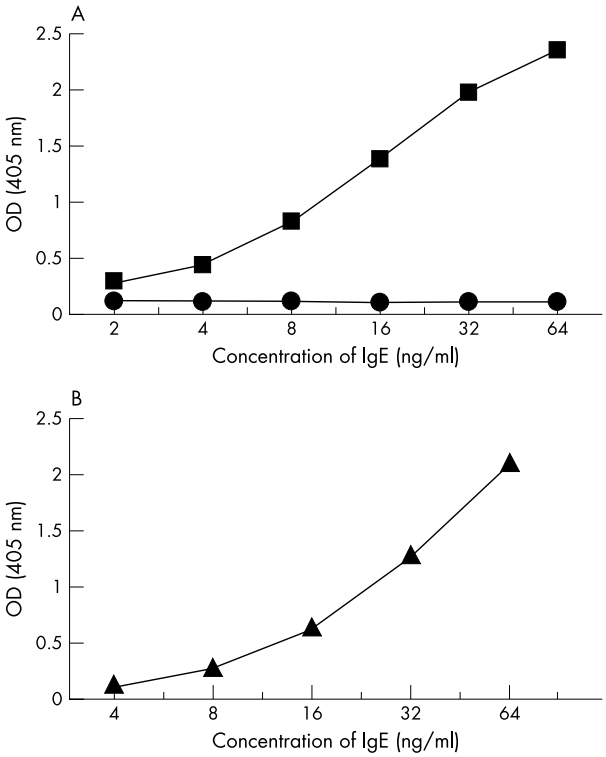

Figure 8 Dose dependent binding of monoclonal antibody (mAb) 2C7huE, from clone 2E3, to Der p 1 captured on to (A) mAb 5H8 or (B) antihuman IgE. Purified mAb 2C7huE was detectable with antihuman Fcε specific antibody (squares) and antihuman κ light chain antibody (triangles), but not with polyclonal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

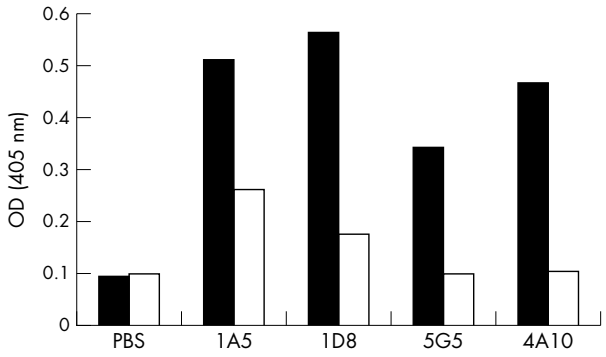

Figure 9 Confirmation of the monoclonal antibody (mAb) 2C7 reactivity and IgG identity of mAb 2G10huG1. The antibodies in cell culture supernatants (100 μl/well) of positive clones 1A5 and 1D8 bound to immobilised mAb 2C7 (open bars) or to immobilised antihuman IgG (solid bars) and were detectable with antihuman κ light chain antibody. These results confirm that mAb 2G10huG1 Vκ and VH chains were correctly assembled. Clones 5G5 and 4A10 containing only VH or Vκ chains belonging to mAb 2G10huG1, respectively, did not recognise mAb 2C7, but as expected were detectable with antihuman κ light chain antibody. This indicates that the complete mAb 2G10 V region is necessary for interaction with mAb 2C7. OD, optical density; PBS, phosphate buffered saline.

2G10huG1, derived from mouse mAb 2C7[17] and mouse mAb 2G10,[19 20] respectively. The newly constructed mAb 2C7huE consists of the original mouse Vκ and VH domains combined with the human Cκ, CH1, and Fcε regions. The newly constructed mAb 2G10huG1 also retained the original mouse Vκ and VH domains combined with the human Cκ, CH1, and Fcγ1 regions.

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-idiotype 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 FcεRI with FcγRIIB

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.[22] 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[40] containing the human constant regions (hucκ or hucγ1), 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 Vκ[22 25 26] and VH[27 28] 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.

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

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. mAb 2C7huE was shown to recognise Der p 1 specifically and also to contain human ε and κ 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 γ and κ constant regions.

We expect mAb 2C7huE and mAb 2G10huG1 to exhibit the effector functions of the appropriate human Ig constant regions; namely, binding to Fcε and Fcγ receptors, respectively. The confirmation that such effector functions are present in the newly constructed chimaeric antibodies should allow us to exploit these reagents to investigate the downregulation of IgE mediated hypersensitivity by the crosslinking of FcεRI with FcγRIIB.[7 13]

ACKNOWLEDGEMENTS

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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