

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-idiotypic

P B Furtado, J E McElveen, L Gough, K L Armour, M R Clark, H F Sewell, F Shakib

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

See end of article for authors' affiliations

Correspondence to:
Dr F Shakib, Division of Molecular and Clinical Immunology, Faculty of Medicine and Health Sciences, Queen's Medical Centre, Nottingham NG7 2UH, UK; farouk.shakib@nottingham.ac.uk

Accepted for publication 21 June 2002

Background: Two mouse monoclonal antibodies have been described, namely: mAb 2C7 (IgG2b κ), which is directed against the major house dust mite allergen Der p 1, and mAb 2G10 (IgG1 κ), which is an anti-idiotypic antibody raised against mAb 2C7. Given its broad IgE specificity, anti-idiotypic 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 Fc ϵ RI bound IgE, and in doing so co-ligating Fc ϵ RI with Fc γ RIIB, 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-idiotypic mAb 2G10 (mAb 2G10huG1).

Methods: The V κ and V H regions of mAb 2C7 and its anti-idiotypic 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-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.

Conclusion: The newly constructed chimaeric antibodies will be useful to investigate the downregulation of IgE mediated hypersensitivity by the crosslinking of Fc ϵ RI with Fc γ RIIB.

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 (Fc ϵ RI), and subsequent crosslinking of Fc ϵ RI bound IgE molecules by allergen leads to the release of the mediators responsible for allergic tissue damage.¹ Fc ϵ RI 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 Fc ϵ RI 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-idiotypic 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 Fc γ RI and Fc γ RIII.^{6,7} ITAM bearing receptors are negatively regulated by an immunoreceptor tyrosine based inhibition motif found on Fc γ RIIB.⁸ Fc γ RIIB, which is highly homologous in mice and humans,⁹ is a single chain low affinity receptor for IgG¹⁰ that is widely distributed on both lymphoid and myeloid cells.¹¹ The negative regulatory effect of Fc γ RIIB is highlighted by the fact that Fc γ RIIB deficient mice show augmented Fc ϵ RI mediated mast cell activation.^{12,13} In keeping with these observations, the crosslinking of Fc ϵ RI with Fc γ RIIB 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

Fc ϵ RI with Fc γ RIIB is thought to be a regulatory physiological event¹⁶ 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 (IgG2b κ), which is directed against the major house dust mite allergen Der p 1,^{17,18} and mAb 2G10 (IgG1 κ), which is an anti-idiotypic antibody raised against mAb 2C7.^{19,20} We have already established that anti-idiotypic mAb 2G10 recognises framework (FRW) residues encoding human immunoglobulin V $\text{H}3$ and V $\text{H}4$ 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-idiotypic 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 Fc ϵ RI bound IgE, and in doing so co-ligating Fc ϵ RI with Fc γ RIIB, 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-idiotypic mAb 2G10 (mAb 2G10huG1).

MATERIALS AND METHODS

Antibody reagents

Mouse anti-Der p 1 mAb 5H8²¹ was obtained from Indoor Biotechnologies Limited (Manchester, UK). A human myeloma

Abbreviations: CDR, complementarity determining region; ELISA, enzyme linked immunosorbent assay; Fc ϵ RI, high affinity IgE receptor; Fc γ RIIB, 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

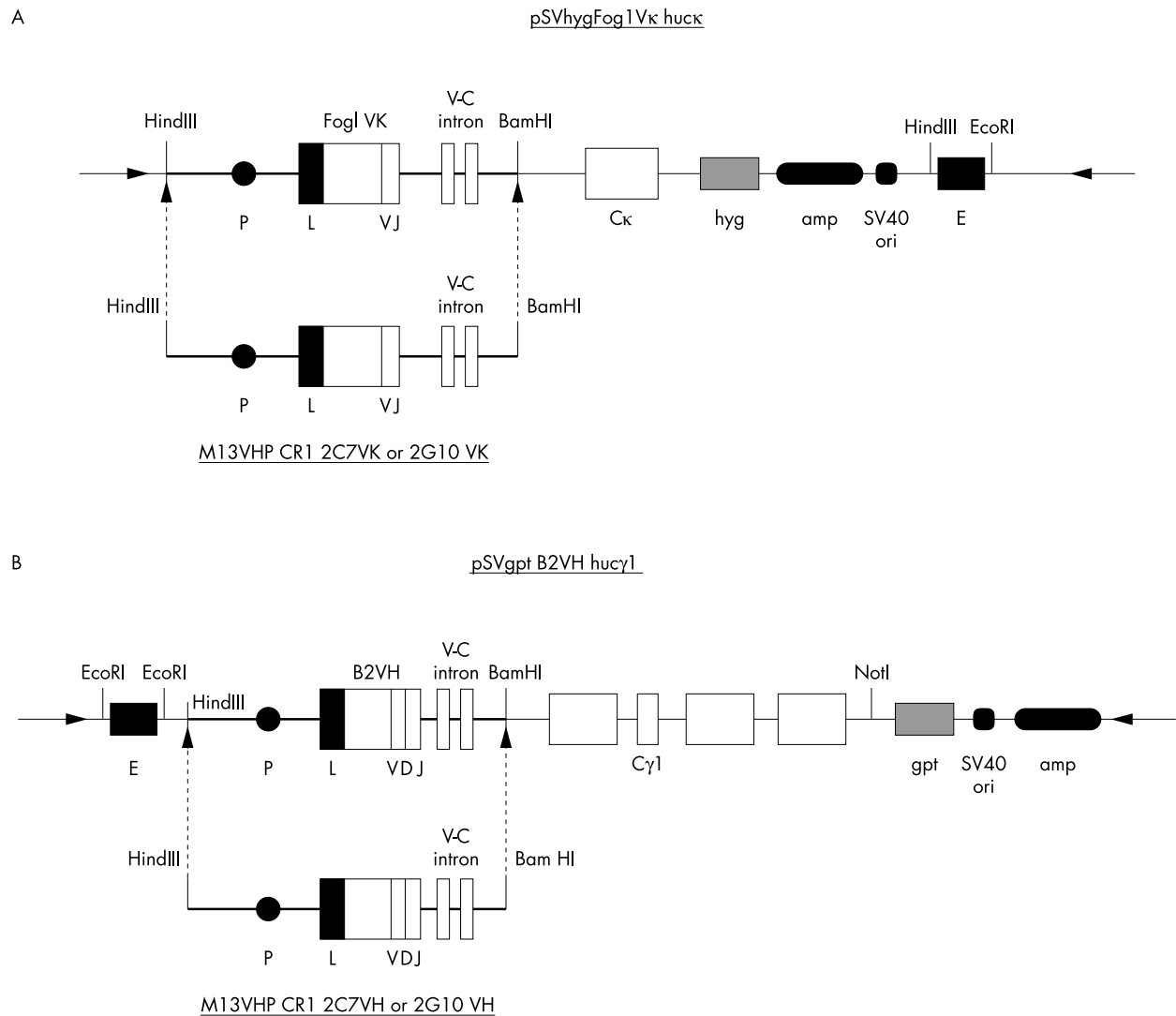


Figure 1 Schematic representation of the chimaerisation of monoclonal antibody (mAb) 2C7 and mAb 2G10 V regions into the huck and hucy1 containing vectors. mAb 2C7 and mAb 2G10 Vκ (VJ) and VH (VDJ) chains were cloned into (A) huck and (B) hucy1 containing vectors, respectively. amp, ampicillin; E, enhancer; 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-idiotypic 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)

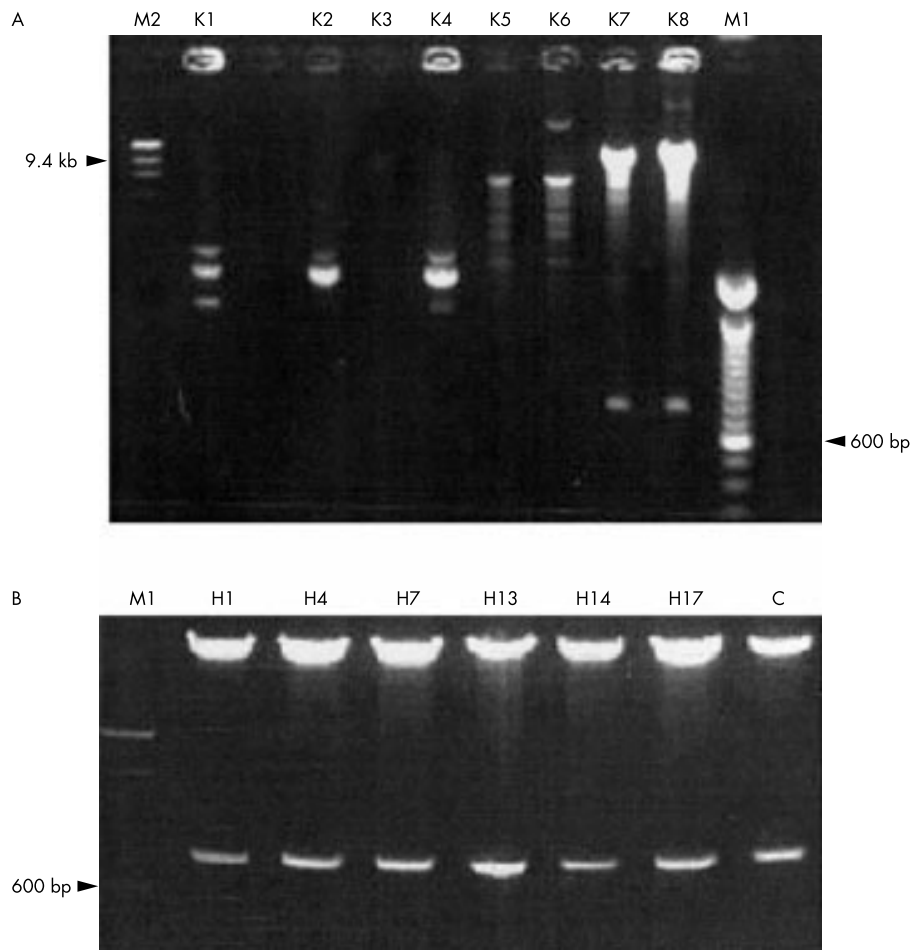


Figure 2 HindIII and BamHI restriction digestion analysis of clones of monoclonal antibody (mAb) 2C7 V region ligated into human constant region containing vectors. Clones containing bands of the expected size for (A) mAb 2C7 V κ (867 bp) and the pSVhyg huc κ vector (10 kb) (K8) and (B) mAb 2C7 VH (890 bp) and the pSVgpt huc γ 1 vector (6.5 kb) (H1–H17) are shown. M1, 100 bp DNA ladder; M2, λ DNA digested with HindIII; C, control (pSVgpt B2VH huc γ 1).

using specifically designed primers (MGW Biotech AG, Ebersberg, Germany). For the mAb 2C7 V κ chain, the oligonucleotides consisted of 2C7VKB (5'-TCCACAGGTGCCACTCCGATGTTGGTGACCCAAAC-3'; coding strand) and 2C7VKF (5'-GAGGTTGTAAGGACTCACCTTTGATTCCAGCTTGG-3'; complementary strand). For the mAb 2C7 VH chain, the oligonucleotides consisted of 2C7VHB (5'-TCCACA GGTGTCCACTCCGAGGTCAGCTGCTCGAGTC-3'; coding strand) and 2C7VHF (5'-GAGGTTGTAAGGACTCACCTGAGGACTGTGAGAG-3'; complementary strand).

Briefly, the procedure involving chimaerisation of mAb 2C7 consisted of exchanging the exons of the cloned mAb 2C7 V κ and VH chains with those from the pSVhyg and pSVgpt vector systems, respectively. The HindIII–BamHI fragment containing the mAb 2C7 V κ and VH chains was cloned into a human constant Ig κ (huc κ)²³ and a human constant IgG1 (huc γ 1), of allotype G1m (1,17),²⁴ by replacement of the V regions of pSVhyg Fog 1 huc κ ^{22 25 26} and pSVgpt B2 huc γ 1,^{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 huc ϵ expression vector was constructed by extraction and cloning of the BamHI fragment containing the huc ϵ region³¹ from the pSV-VNP huc ϵ 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 V κ 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 V κ chain, the oligonucleotides consisted of: 2G10VKB (5'-TCCACAGG TGTCCACTCCAACATTGTGCTGACCCAGTT-3'; coding strand) and 2G10VKF (5'-GAGGTTGTAAGGACTCACCTTTTATTCC AGCCTGGTCCCCCTCCGA-3'; complementary strand). For the mAb 2G10 VH chain, the oligonucleotides consisted of: 2G10VHB2 (5'-TCCACAGGTGCCACTCCCAGGTCCAGTTGCA GCAGTCTGGACC-3'; coding strand) and 2G10VHF (5'-GAG GTTGTAAAGGACTCACCTGAGGAGACGGTGACTG-3'; complementary strand).

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

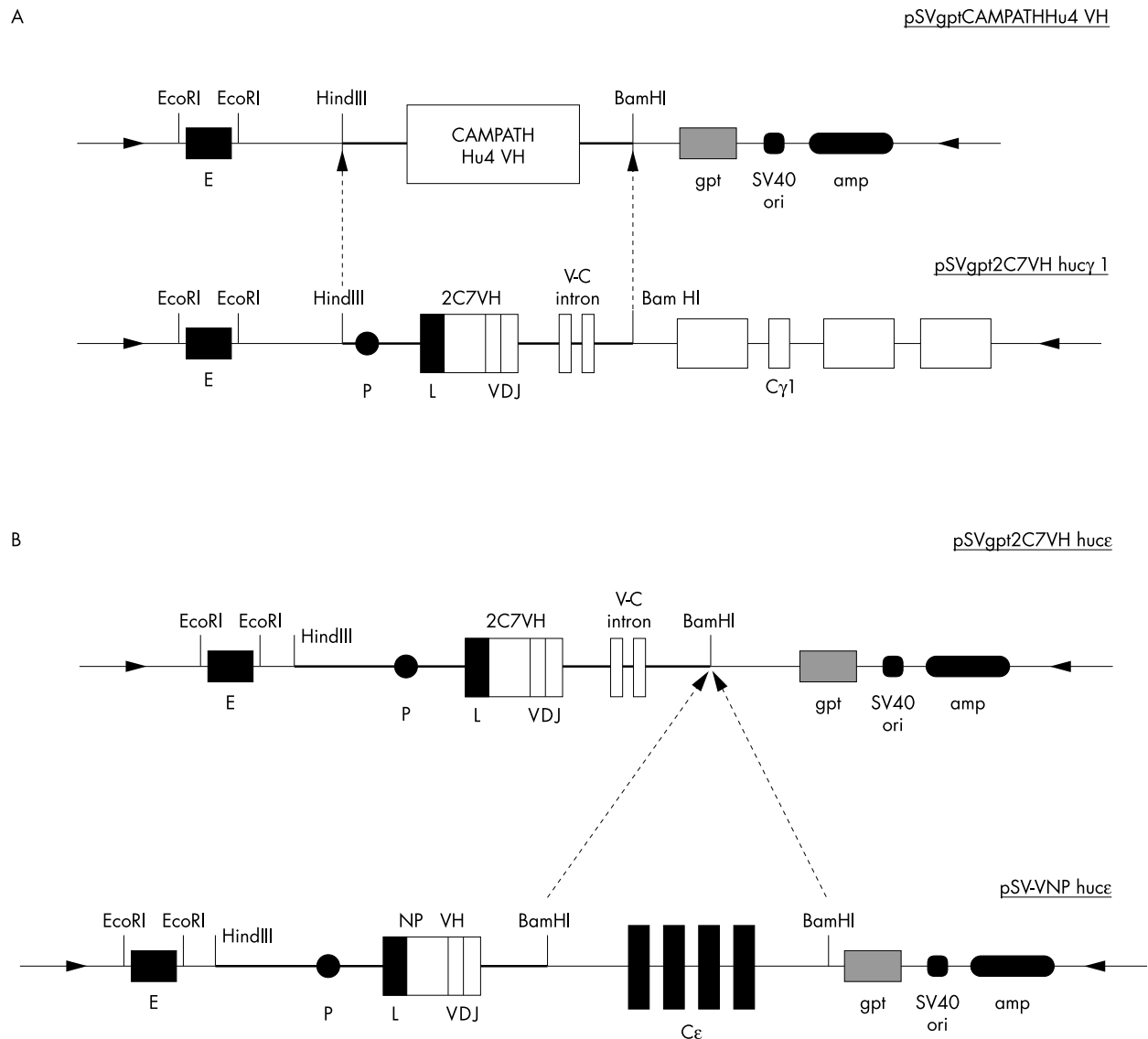


Figure 3 Schematic representation of the production of a chimaeric IgE version of monoclonal antibody (mAb) 2C7 VH. (A) The mAb 2C7 VH chain was cloned into a pSVgpt vector, encoded within a HindIII–BamHI site containing the IgH enhancer (E) (pSVgpt 2C7VH), followed by (B) the full chimaerisation of the mAb 2C7 VH chain to a human IgE version (pSVgpt 2C7VH huce). 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 V κ 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 V κ and VH chains, were cloned into plasmids containing hucy²³ and hucy1, of allotype G1m(1,17),²⁴ by replacement of the V regions of the pSVhyg Fog 1 hucy^{22–25–26} and pSVgpt B2VH hucy1,^{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 μ g of each chimaeric pSVhyg 2C7V κ hucy and pSVhyg 2G10V κ hucy and

10 μ g of each chimaeric pSVgpt 2C7VH hucy1 and pSVgpt 2G10VH hucy1 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 μ g/ml streptomycin, 5mM HEPES (Sigma), and 5% fetal calf serum (FCS) (IMDM complete). Transfectants were selected for resistance to 250 μ g/ml xanthine (Sigma) and 0.8 μ g/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

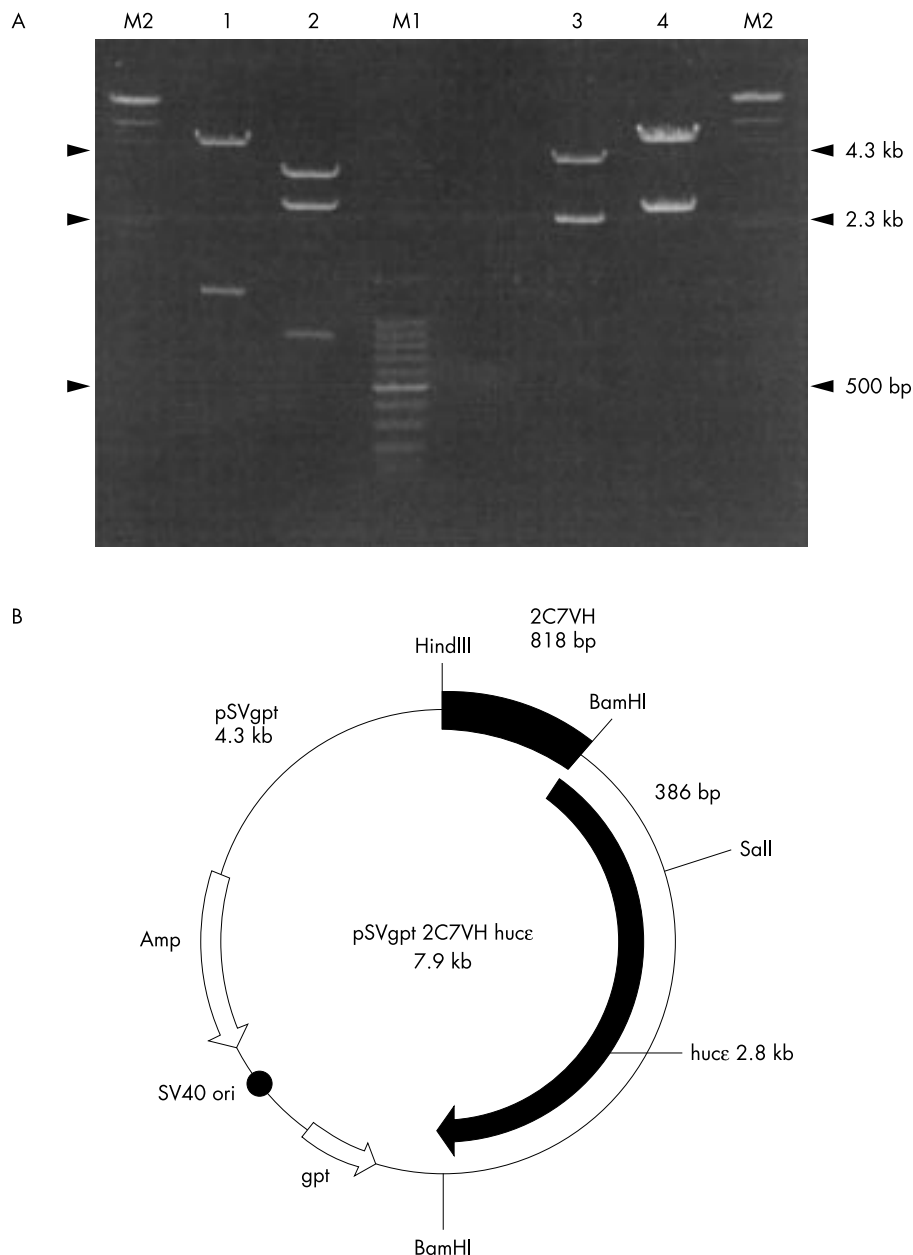


Figure 4 (A) Restriction digests with (1) *Sall* and *HindIII* (6.8 kb and 1.2 kb), (2) *BamHI* and *HindIII* (4.3 kb, 2.8 kb, and 818 bp), (3) *Sall* and *BamHI* (5.1 kb, 2.5 kb, and 386 bp), or (4) just *BamHI* (5.1 kb and 2.8 kb) were used to confirm that (B) the monoclonal antibody 2C7 VH chain had been inserted into the *huε* region containing vector in the correct orientation. M1, 100 bp DNA ladder; M2, λ DNA 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 μ g/ml xanthine and 0.8 μ g/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 μ g/ml)²¹ coated microtitre plates were used to capture Der p 1 (2 μ g/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 μ g/ml) or goat antihuman IgG (1/50 000 dilution; Sigma). The cell culture supernatants of clones 1A5 and 1D8 of mAb 2G10huG1 (100 μ l/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

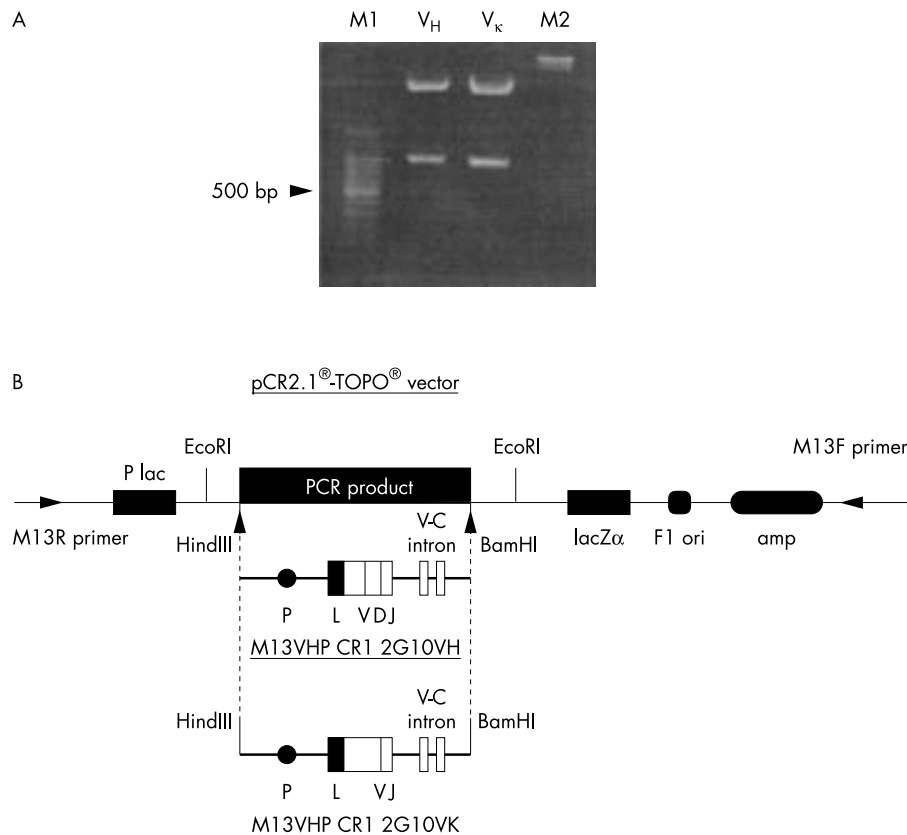


Figure 5 (A) Restriction digestion with EcoRI of clones containing monoclonal antibody 2G10 V κ and VH chains (B) inserted into the pCR2.1-TOPO vector. M1, 100 bp DNA ladder; M2, λ DNA digested with HindIII. amp, ampicillin; L, leader; ori, origin; P, promoter.

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 μ l/well of cell culture supernatant from clones 5G5 (mAb Fog1V κ /2G10VH-IgG1 κ) and 4A10 (mAb 2G10V κ /B2VH-IgG1 κ).

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 κ and VH chains were chimaerised to retain the original murine complementarity determining regions (CDRs) and FRW regions, but with the huc κ /CL and the hucE/CH1 regions, respectively. The chimaerisation of mAb 2C7V κ and VH chains consisted of adding the M13VHPCR1 vector extensions²² 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 V κ and VH chains, with the M13VHPCR1 extensions, were therefore cloned into plasmid vectors containing the huc κ and hucY1 constant regions, respectively (fig 1). Clones containing the pSVhyg huc κ (10 kb) and mAb 2C7 V κ (867 bp) or pSVgpt hucY1 (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 hucE vector,^{32, 34} containing the Fc ϵ 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 hucE vector by replacing the mAb NPVH.³² This vector also provided another IgH enhancer sequence³⁰ to the construct located upstream of the mAb 2C7 VH exons. The human IgE constant region (hucE),³¹ derived from the plasmid vector pSVgpt NP hucE³² (fig 3) was subcloned into the mAb 2C7 VH construct, which resulted in the chimaeric pSVgpt 2C7 VH hucE 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 hucE constant region (fig 4). Similar restriction digests with BamHI and HindIII confirmed that the mAb 2C7 V κ chain has been inserted into the huc κ constant region (fig 2). These results were also confirmed by sequencing the mAb 2C7 V κ 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 V κ and VH were chimaerised to retain the original murine CDRs and FRW regions, but with the huc κ /CL and the hucY1/CH1 regions, respectively. The chimaerisation of the mAb 2G10 V κ and VH chains consisted of adding the M13VHPCR1 vector extensions²² 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 V κ 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 V κ and VH chains were redigested with BamHI and HindIII and cloned into plasmid vectors containing the huc κ

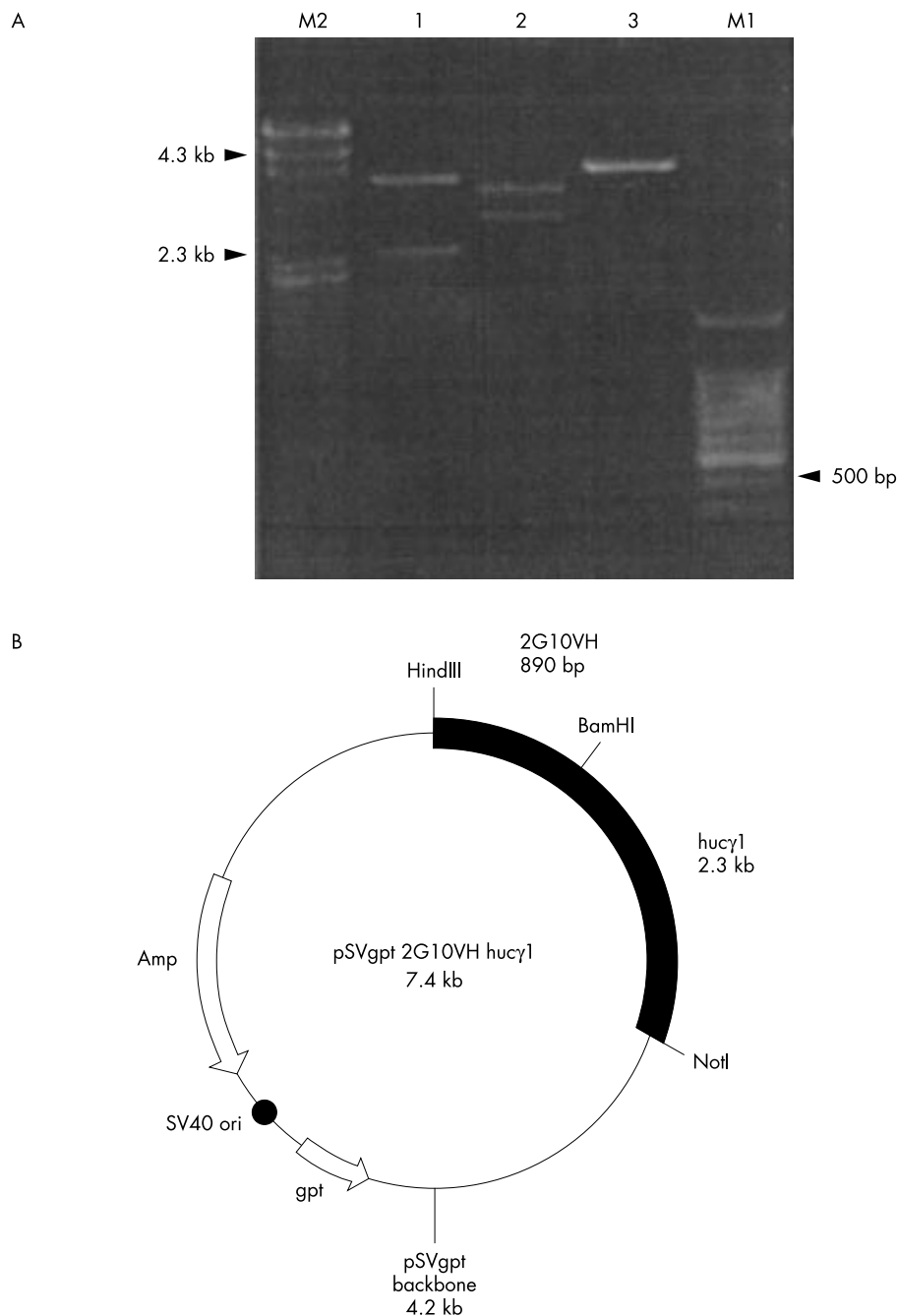


Figure 6 Restriction digests of pSVgpt 2G10VH huc γ 1 with (1) NotI and BamHI (5.0 kb and 2.3 kb), (2) NotI and HindIII (4.2 kb and 3.1 kb), and (3) HindIII and BamHI (6.5 kb and 890 bp) showing that (A) the monoclonal antibody 2G10 VH chain has been inserted into pSVgpt huc γ 1 and (B) the localisation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

and huc γ 1 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 huc γ 1 region (fig 6). Similar restriction digests with EcoRI and HindIII confirmed that the mAb 2C7 V κ chain has been inserted into the constant huc κ region (fig 7). These results were also confirmed by sequencing the mAb 2G10 V κ 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 Fc ϵ 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 IgE κ identity (fig 8).

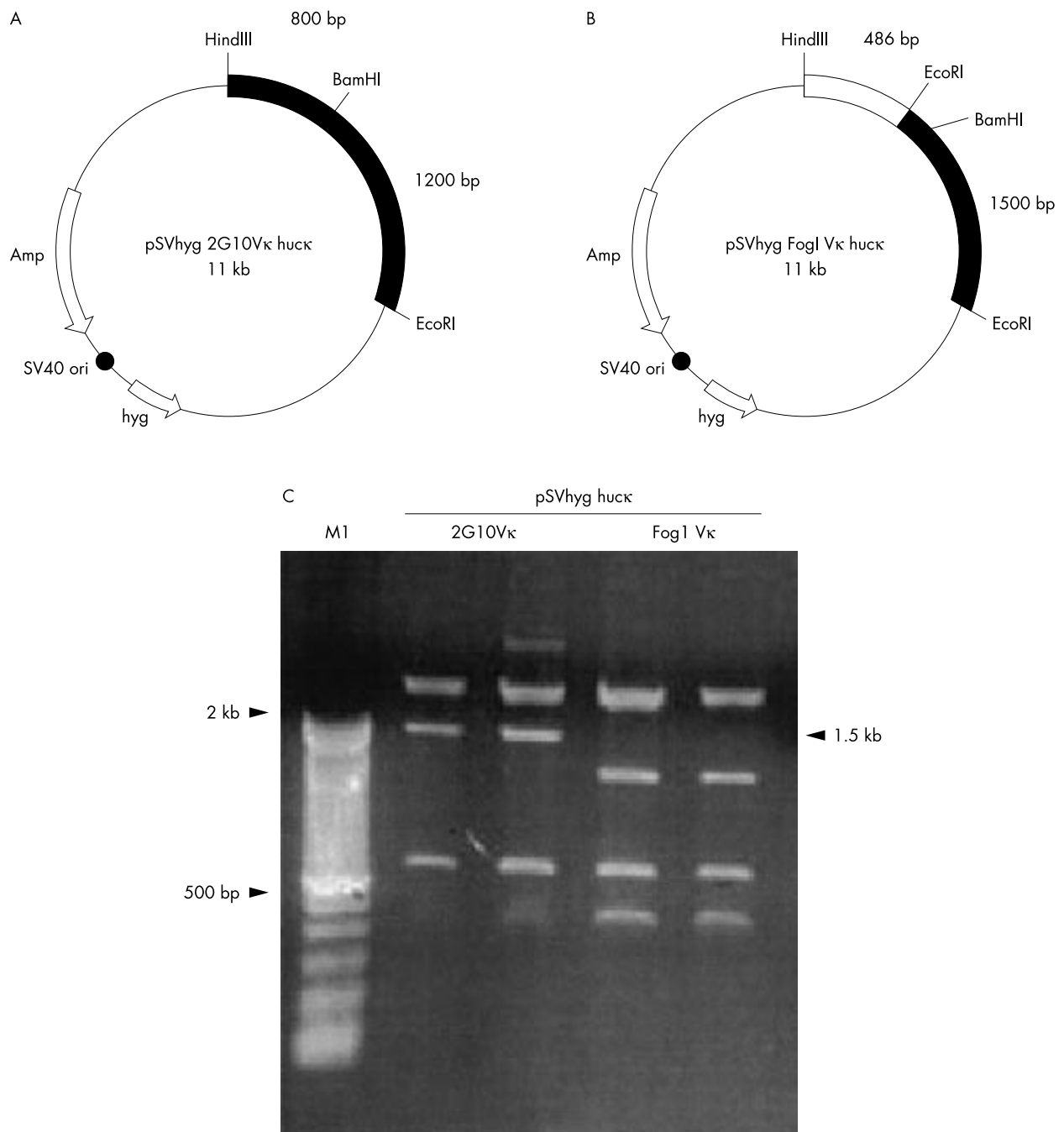


Figure 7 EcoRI and HindIII restriction digestion analysis of transformed clones with the pSVhyg 2G10Vκ huck vector. (A) The monoclonal antibody (mAb) 2G10 Vκ chain is not cut by EcoRI, whereas (B) mAb Fog1Vκ has one EcoRI site at position 468 downstream from the HindIII site. (C) The other released fragments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

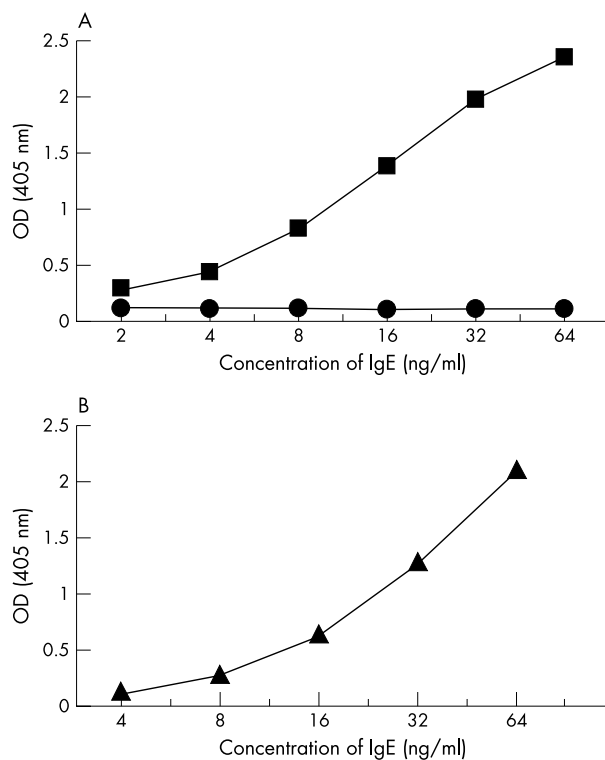


Figure 8 Dose dependent binding of monoclonal antibody (mAb) 2C7huE, from clone 2E3, to Der p 1 captured on to (A) mAb 5H8 or (B) antihuman IgE. Purified mAb 2C7huE was detectable with antihuman Fcε specific antibody (squares) and antihuman κ light chain antibody (triangles), but not with polyclonal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

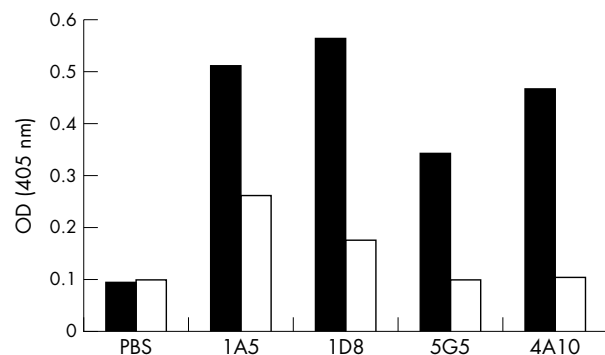


Figure 9 Confirmation of the monoclonal antibody (mAb) 2C7 reactivity and IgG identity of mAb 2G10huG1. The antibodies in cell culture supernatants (100 μl/well) of positive clones 1A5 and 1D8 bound to immobilised mAb 2C7 (open bars) or to immobilised antihuman IgG (solid bars) and were detectable with 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 2C7¹⁷ 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 vectors⁴⁰ containing the human constant regions (huck or hucy1), 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).

.....

Authors' affiliations

P B Furtado, J E McElveen, L Gough, H F Sewell, F Shakib, Division of Molecular and Clinical Immunology, University of Nottingham, Faculty of Medicine and Health Sciences, Queen's Medical Centre, Nottingham NG7 2UH, UK

K L Armour, M R Clark, Immunology Division, Department of Pathology, Cambridge University, Tennis Court Road, Cambridge CB2 1QP, UK

REFERENCES

- Sutton BJ, Gould HJ. The human IgE network. *Nature* 1993;**366**:421–8.
- Blank U, Ra C, Miller L, et al. Complete structure and expression in transfected cells of high affinity IgE receptor. *Nature* 1989;**337**:187–90.
- Kurosaki T, Gander I, Wirthmueller U, et al. The β subunit of the Fc ϵ R1 is associated with the Fc γ R11 on mast cells. *J Exp Med* 1992;**175**:447–51.
- Pleinman C, D'Ambrosio D, Cambier J. The B cell antigen receptor complex: structure and signal transduction. *Immunol Today* 1994;**15**:393–9.
- Weiss A. T cell antigen receptor signal transduction: a tale of tails and cytoplasmic protein-tyrosine kinases. *Cell* 1993;**73**:209–12.
- Lefourneur F, Klausner RD. T cell and basophil activation through the cytoplasmic tail of T cell receptor ζ family proteins. *Proc Natl Acad Sci U S A* 1991;**88**:8905–9.
- Daëron M, Malbec O, Bonnerot S, et al. Tyrosine-containing activation motif-dependent phagocytosis in mast cells. *J Immunol* 1994;**152**:783–92.
- Daëron M, Latour S, Malbec O, et al. The same tyrosine-based inhibition motif, in the intra-cytoplasmic domain of Fc γ R11B, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. *Immunity* 1995;**3**:635–46.
- Brooks DG, Qiu WQ, Luster AD, et al. Structure and expression of human IgG Fc γ R11 (CD32): functional heterogeneity is encoded by the alternatively spliced products of multiple genes. *J Exp Med* 1989;**170**:1369–86.
- Hibbs ML, Walker ID, Kirszenbaum L, et al. The murine Fc receptor for immunoglobulin: purification, partial amino acid sequence, and isolation of cDNA clones. *Proc Natl Acad Sci U S A* 1986;**83**:6980–4.
- Hulett MD, Hogarth PM. Molecular basis of Fc receptor function. *Adv Immunol* 1994;**57**:1–127.
- Takai T, Ono M, Hikida M, et al. Augmented humoral and anaphylactic responses in Fc γ R11-deficient mice. *Nature* 1996;**379**:346–9.
- Ujike A, Ishikawa Y, Ono M, et al. Modulation of immunoglobulin (Ig) E-mediated systemic anaphylaxis by low-affinity Fc receptors for IgG. *J Exp Med* 1999;**189**:1573–9.
- Daëron M, Malbec O, Latour S, et al. Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. *J Clin Invest* 1995;**95**:577–85.
- Malbec O, Fridman WH, Daëron M. Negative regulation of c-kit-mediated cell proliferation by Fc γ R11B. *J Immunol* 1999;**162**:4424–9.
- Malbec O, Fong DC, Turner M, et al. Fc ϵ receptor α -associated lyn-dependent phosphorylation of Fc γ receptor 11B during negative regulation of mast cell activation. *J Immunol* 1998;**160**:1647–58.
- 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.
- Furmanovicene R, Tighe PJ, Clark MR, et al. The use of phage-peptide libraries to define the epitope specificity of a mouse monoclonal anti-Der p 1 antibody representative of a major component of the human immunoglobulin E anti-Der p 1 response. *Clin Exp Allergy* 1999;**29**:1563–71.
- McElveen JE, Furtado PB, Smith SJ, et al. Characterisation of a mouse monoclonal anti-idiotype reactive with a V-region sequence commonly used by human immunoglobulins. *Mol Pathol* 2000;**53**:77–82.
- Furtado PB, Furmanovicene R, McElveen JE, et al. Prediction of the interacting surfaces in a tri-molecular complex formed between the major dust mite allergen Der p 1, a mouse monoclonal anti-Der p 1 antibody and its anti-idiotype. *Mol Pathol* 2000;**53**:324–32.
- 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.
- Orlandi R, Gussow DH, Jones PT, et al. Cloning immunoglobulin variable domains for expression by the polymerase chain reaction. *Proc Natl Acad Sci U S A* 1989;**86**:3833–7.
- Hieter PA, Max EE, Seidman JG, et al. Cloned human and mouse kappa immunoglobulin constant and J region genes conserve homology in functional segments. *Cell* 1980;**22**:197–207.
- Takahashi N, Ueda S, Obata M, et al. Structure of human immunoglobulin gamma genes: implications for evolution of a gene family. *Cell* 1982;**29**:671–9.
- Bye JM, Carter C, Cui Y, et al. Germline variable region gene segment derivation of human monoclonal anti-Rh (D) antibodies. Evidence for affinity maturation by somatic hypermutation and repertoire shift. *J Clin Invest* 1992;**90**:2481–90.
- Armour KL, Clark MR, Hadley AG, et al. Recombinant human IgG molecules lacking Fc γ receptor 1 binding and monocyte triggering activities. *Eur J Immunol* 1999;**29**:2613–24.
- Clark MR. Humanised antibodies having modified determinants (immunoglobulin allotypes). Patent application WO 92/16562, 1992.
- Garner SF, Smethurst PA, Merieux Y, et al. A rapid one-stage whole-blood HPA-1a phenotyping assay using a recombinant monoclonal IgG1 anti-HPA-1a. *Br J Haematol* 2000;**108**:440–7.
- Riechmann L, Clark MR, Waldmann H, et al. Reshaping human antibodies for therapy. *Nature* 1988;**332**:323–7.
- Neuberger MS. Expression and regulation of immunoglobulin heavy chain gene transfected into lymphoid cells. *EMBO J* 1983;**2**:1373–8.
- Flanagan JG, Rabbitts TH. The sequence of a human immunoglobulin epsilon heavy chain constant region gene, evidence for three non-allelic genes. *EMBO J* 1982;**1**:655–60.
- Neuberger MS, Williams GT, Mitchell EB, et al. A hapten-specific chimaeric IgE antibody with human physiological effector function. *Nature* 1985;**314**:268–9.
- Kilmartin JV, Wright B, Milstein C. Rat monoclonal anti-tubulin antibodies derived by using a nonsecreting rat cell line. *J Cell Biol* 1982;**93**:576–82.
- Bruggemann M, Williams GT, Bindon CI, et al. Comparison of the effector functions of human immunoglobulins using a matched set of chimaeric antibodies. *J Exp Med* 1987;**166**:1351–61.
- Winter G, Milstein C. Man-made antibodies. *Nature* 1991;**349**:293–9.
- Carter P, Merchant AM. Engineering antibodies for imaging and therapy. *Curr Opin Biotechnol* 1997;**8**:449–54.
- Vaswani SK, Hamilton RG. Humanized antibodies as potential therapeutic drugs. *Ann Allergy Asthma Immunol* 1998;**81**:105–19.
- Kettleborough CA, Saldanha J, Ansell KH, et al. Optimization of primers for cloning libraries of mouse immunoglobulin genes using the polymerase chain reaction. *Eur J Immunol* 1993;**23**:206–11.
- Coloma JM, Hastings A, Wims LA, et al. Novel vectors for the expression of antibody molecules using variable regions generated by polymerase chain reaction. *J Immunol Methods* 1992;**152**:89–104.
- Mulligan RC, Berg PC. Selection for animal cells that express the Escherichia coli gene coding for xanthine-guanine phosphoribosyl transferase. *Proc Natl Acad Sci U S A* 1981;**78**:2072–6.

New JCP online submission and review system

We are pleased to inform authors and reviewers of the new online submission and review system at JCP. Developed by High-Wire Press (CA, USA), Bench Press is a fully integrated electronic system that utilises the web to allow rapid and efficient submission of manuscripts. It also allows the peer review process to be conducted entirely online. We are one of the first journals in the BMJ Special Journals group to go online in this way. The aim, apart from saving trees, is to speed up the often frustratingly slow process (for both authors and editors) from submission to publication. Many reviewers might appreciate this too. Authors may submit their manuscript in any standard word processing software. Acceptable standard graphic formats include: jpeg, tiff, gif, and eps. The text and graphic files are automatically converted to PDF for ease of distribution and reviewing purposes. Authors are asked to approve their submission before it formally enters the reviewing process. On approval by the authors, the submission is passed to the editor and/or reviewers via the web. All transactions are secure.

To access the system click on "SUBMIT YOUR MANUSCRIPT HERE" on the JCP homepage: HYPERLINK <http://www.jclinpath.com>, or you can access Bench Press directly at HYPERLINK <http://submit-jcp.bmjournals.com>.

We are very excited with this new development and would encourage authors and reviewers to use the online system whenever possible. As editors, we will use it all the time, the up side being lack of need to travel to the editorial office to deal with papers, the down side having no more excuses to postpone decisions on papers because we are "at a meeting"!

The system is very easy to use and should be a big improvement on the current peer review process. Full instructions can be found on Bench Press <http://submit-jcp.bmjournals.com> and JCP online at <http://www.jclinpath.com>. Please contact Natalie Davies, Project Manager, HYPERLINK <mailto:ndavies@bmjgroup.com> for any further information.

H Holzel, P van Diest



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

P B Furtado, J E McElveen, L Gough, et al.

Mol Pathol 2002 55: 315-324

doi: 10.1136/mp.55.5.315

Updated information and services can be found at:

<http://mp.bmj.com/content/55/5/315.full.html>

References

These include:

This article cites 37 articles, 15 of which can be accessed free at:

<http://mp.bmj.com/content/55/5/315.full.html#ref-list-1>

Article cited in:

<http://mp.bmj.com/content/55/5/315.full.html#related-urls>

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:

<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:

<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:

<http://group.bmj.com/subscribe/>