

Humanization of a mouse anti-human IgE antibody: a potential therapeutic for IgE-mediated allergies

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Mouse mAb TES-C21(C21) recognizes an epitope on human IgE and, therefore, has potential as a therapeutic agent in patients with IgE-mediated allergies such as hay fever, food and drug allergies and extrinsic asthma. The clinical usefulness of mouse antibodies is limited, however, due to their immunogenicity in humans. Mouse C21 antibody was humanized by complementarity determining region (CDR) grafting with the aim of developing an effective and safe therapeutic for the treatment of IgE-mediated allergies. The CDR-grafted, or reshaped human, C21 variable regions were carefully designed using a specially constructed molecular model of the mouse C21 variable regions. A key step in the design of reshaped human variable regions is the selection of the human framework regions (FRs) to serve as the backbones of the reshaped human variable regions. Two approaches to the selection of human FRs were tested: (i) selection from human consensus sequences and (ii) selection from individual human antibodies. The reshaped human and mouse C21 antibodies were tested and compared using a biosensor to measure the kinetics of binding to human IgE. Surprisingly, a few of the reshaped human C21 antibodies exhibited patterns of binding and affinities that were essentially identical to those of mouse C21 antibody.

Key words: antibody/biosensor/CDR grafting/human IgE/molecular modelling

Introduction

Mouse mAb TES-C21 (C21) was isolated from mice immunized with polyclonal IgE purified from human serum (Davis *et al.*, 1993). Mouse mAb C21 binds to secreted human IgE circulating in plasma and to the membrane-anchored IgE present on the surface of IgE-expressing B cells, but does not interact with IgE when it is bound to its low- or high-affinity receptors (FcεRI and FcεRII respectively) on mast cells, basophils and other cells. Mouse mAb C21, therefore, does not induce mast cells and basophils to release histamine and other mediators that cause allergic symptoms. Antibodies that recognize this very defined region of human IgE may be useful and safe for clearing circulating IgE from the blood and for specifically targeting IgE-secreting B cells, but not other cells bearing IgE. These antibodies, therefore, may have therapeutic applications in the treatment of IgE-mediated allergies (Chang *et al.*, 1990).

The development and use of mouse mAbs as therapeutic agents have been hindered by the human anti-mouse antibody response

(HAMA) which reduces the half-life and, therefore, the efficacy of the mouse antibody in patients (see review by Adair *et al.*, 1990). In addition, there are risks of adverse side-effects associated with repeated administrations of highly immunogenic foreign protein to patients. Many of the problems associated with the use of mouse mAbs as therapeutic agents could be overcome with the use of human mAbs. It has proven technically difficult, however, to isolate the latter. In addition, it would be particularly difficult to isolate high-affinity human antibodies recognizing self-antigens such as human IgE. In order to make mouse monoclonal antibodies more acceptable as therapeutic agents, a variety of approaches have been developed for rendering mouse mAbs less immunogenic in humans by making them resemble human antibodies. Most approaches focus on replacing parts of the mouse antibody with parts of human antibodies (see review by Presta, 1992). The most complete method for 'humanization' consists of taking only the complementarity determining regions (CDRs) from the mouse antibody variable regions and grafting these mouse CDRs into human variable regions (Jones *et al.*, 1986). The CDR-grafted variable regions, or reshaped human variable regions, are then joined to human constant regions to create a reshaped human antibody. This study describes the successful humanization of mouse C21 antibody by CDR grafting.

Materials and methods

Molecular modelling of the mouse C21 variable regions

The DNA sequences of the variable regions of mouse mAb C21 were provided by Tanox Biosystems Inc. (Houston, TX). A 3-D model of the variable regions was built based on protein sequences derived from the DNA sequences. The model was developed on a Silicon Graphics IRIS 4D workstation using the molecular modelling package QUANTA (Polygen Corporation, Waltham, MA). The light chain variable region (V_L) was modelled on the structure of the mouse anti-lysozyme antibody HyHEL-10 as solved by X-ray crystallography (Padlan *et al.*, 1989). The heavy chain variable region (V_H) was modelled on the structure of the mouse anti-lysozyme antibody HyHEL-5 (Sheriff *et al.*, 1987). The V_L and V_H regions of mouse C21 antibody have 79 and 80% identity, respectively, to mouse HyHEL-10 and HyHEL-5 antibodies. Identical residues in the framework regions (FRs) were retained and non-identical residues were substituted using QUANTA. CDR1, CDR2 and CDR3 of the V_L region and CDR1 and CDR2 of the V_H region from mouse C21 antibody corresponded well to the canonical forms postulated by Chothia *et al.* (1989). Minor variations from the canonical sequences were seen, however, at residue 33 in CDR1 of the V_L region and residue 55 in CDR2 of the V_H region. The main chain torsion angles of these loops were the same as those of the original antibody structures (HyHEL-10 for CDR1, CDR2 and CDR3 of the V_L region; HyHEL-5 for CDR1 and CDR2 of the V_H region). Because there are no canonical structures for CDR3s of V_H regions, CDR3 of the V_H region of mouse C21 antibody was modelled on a loop selected from 91 high-resolution protein structures in the Brookhaven Databank (Bernstein *et al.*, 1977).

Thirty candidate loops were extracted using the algorithm of Jones and Thirup (1986) as implemented in QUANTA. The best loops were selected by eye. The loops were anchored on three amino acid residues in the FRs on either side of the CDR3 in the mouse C21 V_H region. The CDR3 in the mouse C21 V_H region was modelled on residues 87–106 of the Bence-Jones protein RHE (Furey et al., 1983). This region of RHE corresponds approximately to the CDR3 of a V_L region. The model was subjected to steepest descents and conjugate gradients energy minimization using the CHARMM potential (Brooks et al., 1983), as implemented in QUANTA, to relieve unfavourable atomic contacts and to optimize van der Waals and electrostatic interactions.

Construction of the reshaped human C21 V_L and V_H regions

The first versions of reshaped human C21 V_L and V_H regions (L1 and H1) were constructed by gene synthesis using six overlapping synthetic DNA oligonucleotides for each construction (Table I, panel A; Table II, panel A). In each case, the six 5'-phosphorylated and PAGE-purified oligonucleotides (Genosys Biotchnologies, Houston, TX) were assembled using a PCR-based protocol. Aliquots of each oligonucleotide (5 pmol) were annealed and extended in a 100 μl reaction containing 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 50 mM KCl, 10 mM β-mercaptoethanol, 0.05% (w/v) Tween-20, 0.05% NP-40, 200 μM dNTPs and 5 U Vent DNA polymerase (New England Biolabs, Beverly, MA). Following one cycle at 95°C for 1 min, 50°C for 2 min and 72°C for 4 min in a Techne PHC-2 temperature cycler, 50 pmol of oligonucleotide primers, designed to hybridize at the 5'- and 3'-ends of the full-length DNA fragment,

Table I. Oligonucleotides used for the construction of the reshaped human C21 V_L regions

Panel A. Oligonucleotides for the synthesis of version L1 of reshaped human C21 V_L region

Olgo 1: C21L1
5'-TGAAGAAAGC TTGCCGCCAC CATGGAGACC CCGGCCAGC TGCTGTCTCT
GCTGCTGCTG TGGCTGCCG ACACCCAGCG CGACATCTCT CTGACCCAGA GCGCC

Olgo 2: C21L2
5'-GATGTTGGTG CCGATGCTCT GGCTGGCCCT GCAGCTCAGG GTGGCCCTCT
CGCCGGGGCT CAGGCTCAGG GTGCCGGGCG TCTGGGTCAG CAGGA

Olgo 3: C21L3
5'-CAGAGCATCG GCACCAACAT CCACTGGTAC CAGCAGAAGC CCGGCCAGCC
CCCCAGGGCT CTGATCAAGT ACGCC

Olgo 4: C21L4
5'-AGGGTGAAGT CCGTGCCTGT GCCGTGCGC CTGAACCTGC TGGGGATGCC
GCTGATGCTC TCGTGGCTT ACTTGATCAG CAGCCTG

Olgo 5: C21L5
5'-GCGCACCGA CTTCCACCTG ACCATCAGCA GGCTGGAGCC CGAGGACTTC
GCCATGACT ACTGCCAGCA GAGCGACAGC TGGC

Olgo 6: C21L6
5'-TTTGGATCCT TCTAGAATC TCACGTTTGA TCTCCACTT GGTGCCCTGG
CGAAGGTGG TGGCCAGCT GTGCTCTGC TG

5' Primer: C21-5'
5'-TGAAGAAAGC TTGCCGCCAC C

3' Primer: C21-L3'
5'-TTTGGATCCT TCTAGAATC TCAC

Panel B. Oligonucleotides for the subsequent construction of versions L2 and L3 of the reshaped human C21 V_L region

Primer L/D60S-L (introduces D60S into L1, complementary strand)
5'-CTGAACCTGT GGGGATGCC GCTGATGCTC

Primer L/D60S-SL (introduces D60S into L1, coding strand)
5'-CCGACAGGT TCAGCGGAC CGCA

Primer L/EID-V3L (introduces EID and V3L into L1, complementary strand)
5'-GGTCAGCAGG ATCTCCCGGG TG

Primer L/EID-V3L-SL (introduces EID and V3L into L1, coding strand)
5'-GAGATCTGC TGACCCAGAG CCGCGC

were added (C21-5', and C21-L3' or C21-H3', Tables I and II, panels A). Then the full-length DNA fragment was amplified in 20 cycles at 95°C for 1 min, 60°C for 2 min and 72°C for 2 min. Next the reaction was chloroform-extracted. The DNA was ethanol-precipitated, digested with *Hind*III and *Bam*HI, and fragments of the correct size purified from an agarose gel. The *Hind*III–*Bam*HI DNA fragments were cloned into a pBluescript KS+ vector (Stratagene, La Jolla, CA) and sequenced using Sequenase (United States Biochemical Corporation, Cleveland, OH). Point mutations and/or deletions within the DNA sequence were corrected by exchanging DNA restriction enzyme fragments between different clones and/or using PCR-based mutagenesis methods (Kammann et al., 1989). *Hind*III–*Bam*HI fragments exhibiting the correct DNA sequences were then subcloned into

Table II. Oligonucleotides used for the construction of the reshaped human C21 V_H regions

Panel A. Oligonucleotides for the synthesis of version H1 of reshaped human C21 V_H region

Olgo 1: C21H1
5'-TGAAGAAAGC TTGCCGCCAC CATGGACTGG ACCTGGAGGG TGTTCGCCT
GCTGCCCTG GCGCCCGGG CCCACAGCA GGTGCAGCTG GTGCAGA

Olgo 2: C21H2
5'-CAGCCAGTAC ATGCTGAAGG TGTAGCCGT GCGCTTGCAG CTCACCTCA
CGCTGGCGCC GGGCTTCTTC ACCTGGGGC CCGCTGCAC CAGCTGCACC TGG

Olgo 3: C21H3
5'-CACCTTCAGC ATGCTACTGGC TGGAGTGGGT GAAGCAGAGG CCGGCCACGG
GCCTGGAGTG GGTGGGGGAG ATCAGCCCGG GCACCTTCAC CACCAACTAC AACGA

Olgo 4: C21H4
5'-GTCCTCGCTG GTCAGGCTGC TCAGCTCCAT GTAGGGGGTG TTGGTGTGG
TGTGGCGGT GAAGGTGGCC TTGGCCCTGA ACTTCTCGTT GTAGTTGGTG GTGAAGG

Olgo 5: C21H5
5'-AGCAGCCTGA CCAGCGGAGGA CACCGCCGTG TACTACTGCG CCAGGTTTCC
CCACTTCAGC GGCAGCAACT ACGACTACTT CGA

Olgo 6: C21H6
5'-TTTGGATCCT TCTAGAATC ACCTGAGCTC ACGGTACCA GGGTGCCTCG
GCCCACTAGT TCGAAGTAGT CGTAGTTGCT GGC

5' Primer: C21-5'
5'-TGAAGAAAGC TTGCCGCCAC C

3' Primer: C21-H3'
5'-TTTGGATCCT TCTAGAATC ACC

Panel B. Oligonucleotides for the subsequent construction of versions H3, Hay1, and Hay3 of the reshaped human C21 V_H region

Primer H/R38K-A40R-L (introduces R38K and A40R into H1, complementary strand)
5'-CCGGGGGCT GCGTCAACCA CTCAGCC

Primer H/R38K-A40R-SL (introduces R38K and A40R into H1, coding strand)
5'-GAGCAGGGC CCGGCCACG GCCTGGAGT

Primer H/R66K-L (introduces R66K into H1, complementary strand)
5'-GAAGGTGGCC GTGGCCCTGA ACTCTCGTT GTAG

Primer H/R66K-SL (introduces R66K into H1, coding strand)
5'-CAAGGCCAG GGCACCTTCA CCGCCGAC

Primer H/R83T-L (introduces R83T into H1, complementary strand)
5'-GTCCTCGCTG CTCAGGCTGC TCAGCTCCAT G

Primer H/R83T-S (introduces R83T into H1 gene, coding strand)
5'-CAGCCTGAGG AGCGAGACA C

Primer HayFR2 (FR2 changes from H1 to Hay1, complementary strand)
5'-CCAATCACTC CAGGCTCTGG CCGGGC

Primer HayFR2-L (FR2 changes from H3 to Hay3, complementary strand)
5'-CCAATCACTC CAGGCTCTGG CCGGGGCT GCG

Primer HayFR2-S (FR2 changes from H1 to Hay1 and H3 to Hay3, coding strand)
5'-CAGAGGCTGG AGTGGATGGG CGAGATC

Primer HayFR3 (FR3 changes from H1 to Hay1 and H3 to Hay3, complementary strand)
5'-GTGCTGGGGC TGGTGTGGG

Primer HayFR3-S (FR3 changes from H1 to Hay1 and H3 to Hay3, coding strand)
5'-ACCAGGCGCA GCACCCCTA C

vectors designed to express human κ light chains or human γ -1 heavy chains in mammalian cells (Maeda *et al.*, 1991).

Additional versions of reshaped human C21 V_L regions (L2 and L3) were generated from version L1 by oligonucleotide-directed PCR mutagenesis. The PCR primers used to create versions L2 and L3 from version L1 are listed in Table I, panel B. Similarly, additional versions of reshaped human C21 V_H regions (H3, Hay1 and Hay3) were generated from version H1 by oligonucleotide-directed PCR mutagenesis. The PCR primers used to create the new versions of reshaped human C21 V_H regions are listed in Table II, panel B. The resulting *Hind*III–*Bam*HI fragments were cloned, sequenced and subcloned into the expression vectors as described previously.

Expression of reshaped human C21 antibodies in *cos* cells

The *cos* cells were co-transfected by electroporation with the plasmid DNAs designed to express the reshaped human C21 light and heavy chains (Maeda *et al.*, 1991). After a 10 min recovery period, the cells were plated out in 10 ml Dulbecco's minimal essential medium containing 5% γ globulin-free, heat-inactivated fetal calf serum. After 72 h incubation, the medium was collected and centrifuged to remove cells and cellular debris. The supernatant was filtered through a 0.45 μ m membrane and analysed by ELISA for assembled antibody with human κ light chains and human γ heavy chains.

Protein A purification of the reshaped human C21 antibodies

Reshaped human C21 antibodies were purified from the *cos* cell supernatants by affinity chromatography on 1 ml immobilized protein A (Prosep A, Bioprocessing Ltd, Durham, UK) packed into HR 5/5 FPLC columns (Pharmacia, Uppsala, Sweden). The columns were run at constant flow rates of 2 ml/min on an FPLC system (Pharmacia). Eluted protein was detected in a flow cell (UV absorbency at 280 nm). The columns were prepared by washing in 10 column volumes of PBS (20 mM sodium phosphate, 150 mM NaCl, pH 8.0), pre-elution with 10 column volumes of 100 mM sodium citrate buffer (pH 3.0) and re-equilibration with 10 column volumes of PBS (pH 8.0). The *cos* cell supernatants (20–50 ml) were clarified by filtration through a 0.45 μ m membrane and then loaded directly onto the column with a peristaltic pump. The column was washed with PBS (pH 8.0) until the UV absorbency returned to baseline. Bovine IgG was then eluted by washing with 100 mM sodium citrate buffer (pH 5.0) until the baseline returned to zero. Finally, reshaped human antibodies were eluted with 100 mM sodium citrate buffer (pH 3.0). The pH was adjusted immediately to pH 7.0 with 1 M Tris. The neutralized eluates containing the reshaped human antibodies were concentrated in a Centricon-10 microconcentrator (Amicon, Stonehouse, UK) and the buffer changed to PBS (pH 7.2). Purity of the reshaped human antibodies was analysed by SDS–PAGE and Coomassie blue staining (Laemmli, 1970). Protein concentration was determined by UV absorption at 280 nm and by ELISA.

ELISA for human γ/κ antibody

Microtiter 96-well plates were coated with goat anti-human IgG (Fc specific) (Dianova). After washing, the plates were blocked with 1% bovine serum albumin in PBS (pH 7.2) plus 0.05% Tween. Sample and sample dilutions were added and, after incubation and washing, bound human IgG/ κ antibody was detected using affinity-purified goat anti-human κ light chain polyclonal antibody conjugated with horseradish peroxidase (Sigma, Poole, UK). A purified recombinant human antibody (human IgG1/ κ) of known concentration was used as a standard.

Analysis of the mouse, chimeric and reshaped human C21 antibodies by Biospecific Interaction Analysis (BIA)

A biosensor-based analytical system (Pharmacia BIAcore) was used to analyse the kinetics of interaction between the C21 antibodies and their antigen, human IgE. Mouse C21 antibody (TES-C21), chimeric C21 antibody (TESC-2) and mouse–human chimeric IgE antibody (SE44, Sun *et al.*, 1991) were provided by Tanox Biosystems Inc. As capture antibodies, ~11,000 resonance units (RU) (11 ng/mm²) of polyclonal rabbit anti-mouse IgG1 (Pharmacia Biosensor AB, Freiburg, Germany) or rabbit anti-human IgG (kindly donated by Dr U. Roder, Pharmacia Biosensor AB) were immobilized to the CM5 sensor chip surface via their amino groups (Jönsson *et al.*, 1991). For each C21 test antibody, four experimental cycles were performed. Each cycle consisted of binding a constant amount of test C21 antibody to the respective capture antibody followed by the interaction of this test C21 antibody with fixed concentrations of human IgE. The assays were carried out at 25°C. Test C21 antibody was diluted in HBS (10 mM HEPES, 3.4 mM EDTA, 150 mM NaCl, 0.05% BIASurfactant, pH 7.4) to a final concentration of 5–10 μ g/ml and bound to catching antibody to obtain 1300–2200 RU (1.3–2.2 ng/mm²) of bound test antibody. Human IgE, at concentrations of 3.125, 6.25, 12.5 and 25 nM, was passed over the bound test C21 antibody at a flow rate of 5 μ l/min for 9 min. An aliquot of 4 μ l of 40 mM HCl was used to remove antibody–antigen complexes and to prepare the surface for the next cycle. The surface plasmon resonance (SPR) signals were measured and illustrated as a sensorgram. The rates of association for the antibody–antigen interactions were calculated using computer programs implemented in the BIAcore system.

For the determination of the rates of dissociation, a similar protocol was used. Test C21 antibodies were first bound to the sensor chip surface via the immobilized capture antibodies. Human IgE (25 nM) was allowed to bind to the C21 test antibody. Then HBS buffer was passed over the sensor chip surface at a constant flow rate of 5 μ l/min and the decrease in resonance signal monitored over a period of 15–25 min. The sensor chip surface was later regenerated with 4 μ l 40 mM HCl. Because the dissociation of antibody–antigen complexes is a first order reaction, the linear parts of the sensorgrams were used to calculate the rates of dissociation using computer programs implemented in the BIAcore system.

The specificities of mouse, chimeric and reshaped human C21 antibodies for human IgE were also tested using the Pharmacia BIAcore machine. The test C21 antibodies were bound to immobilized capture antibodies on the sensor chip surface as described previously. Human Igs with κ light chains and a variety of heavy chains (IgM, IgD; Serotec, Oxford, UK) (IgA1, IgA2; Calbiochem, Nottingham, UK) (IgG4, IgG3, IgG2, IgG1; Sigma) (IgE; Tanox Biosystems Inc.) were passed over the surface at concentrations of 5 μ g/ml. The SPR signals were measured and illustrated as sensorgrams.

Results

Molecular model of the structure of the mouse C21 variable regions

To design reshaped human variable regions that recreate as closely as possible the antigen binding site in the original mouse antibody, it would be useful to know the structure of the mouse Ig variable regions (Verhoeyen *et al.*, 1988). In most cases, however, the structure of the mouse antibody to be humanized

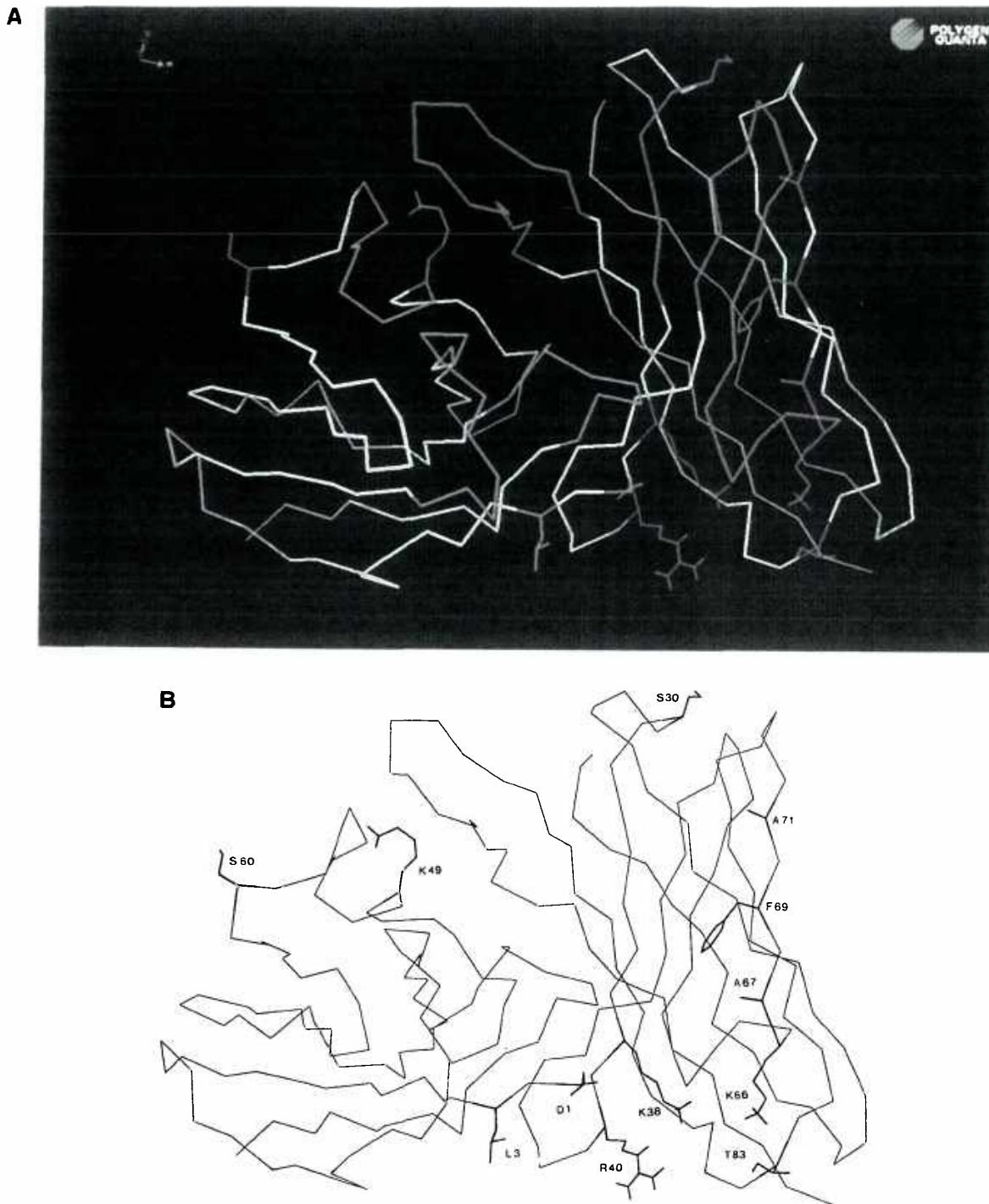


Fig. 1. A view of the molecular model of the variable regions of mouse C21 antibody. (A) The α trace of the variable regions with the FRs in yellow, the CDRs in the V_L region in blue, the CDRs in the V_H region in green, residues of special interest in the FRs of the V_L region in purple, and residues of special interest in the FRs of the V_H region in red. (B) A line drawing of (A) with the residues of special interest labelled.

has not, as yet, been determined. In these cases, a molecular model of the mouse antibody can be constructed as a guide to the design of the reshaped human variable regions (Kettleborough

et al., 1991). In preparation for the design of the reshaped human C21 variable regions, a molecular model of the V_L and V_H regions of mouse C21 antibody was built. Details of the

	FR1		CDR1	FR2	CDR2
	1	2	3	4	5
	12345678901234567890123	45678901234	5678901234	567890123456789	0123456
	*		*****	*	***
C21	DILLTQSPAILSVPGERVSPFC	RASQSIGTNIH	WYQRTDGSPrLLIK	YASESIS	
SGIII	EIVLTQSPGTLSPGERATLSC		WYQKPGQAPRLLIY		
KAF	EIVLTQSPGTLSPGERATLSC		WYQKPGQAPRLLI <u>S</u>		
L1	D-L-----	RASQSIGTNIH	-----	K	YASESIS
L2	D-L-----	-----	-----	K	-----
L3	-----	-----	-----	K	-----

	FR3		CDR3	FR4	
	6	7	8	9	10
	7890123456789012345678	90123456	78901234567		
	*	*	*****		
C21	GIPSRFSGSGSGTEFTLNINSVESEDIADYYC	QQSDSWPTT	FGGGTKLEIK		
SGIII	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC		FGQGTKVEIK		
KAF	GIPDRFSGSGSGTDFTLTISRLEPEDFAMYYC		FGQGTKVEIK		
L1	---S-----	QQSDSWPTT	-----		
L2	-----	-----	-----		
L3	---S-----	-----	-----		

Fig. 2. Comparisons of the amino acid sequences of mouse and reshaped human C21 V_L regions. C21 shows the FRs and CDRs of the mouse C21 V_L region. The amino acid residues that are part of the canonical sequences for the CDR loop structures are marked with an asterisk (Chothia *et al.*, 1989). The numbering is according to Kabat *et al.* (1987). SGIII shows the FRs of the consensus sequence for human $\times V_L$ regions of subgroup III (Kabat *et al.*, 1987). KAF shows the FRs from the V_L region of human KAF antibody (Newkirk *et al.*, 1988). The residues in the FRs of KAF that differ from those in the consensus sequence are underlined. L1, L2 and L3 are the versions of reshaped human C21 V_L region. The residues in the FRs that differ from the KAF sequence are shown in bold.

construction of the model are described in Materials and methods. A view of the model highlighting the amino acid residues that were of particular interest is shown in Figure 1.

Design of the reshaped human C21 variable regions

The design of the reshaped human C21 V_L and V_H regions was based on either the consensus sequences for certain subgroups of human V_L and V_H regions (Kabat *et al.*, 1987) or the sequences from individual human antibodies. The amino acid sequences of the mouse C21 V_L and V_H regions were most similar to the consensus sequence for human $\times V_L$ subgroup III (69% identity within the FRs) and for human V_H subgroup I (70% identity within the FRs). In the first step of the design process, the mouse C21 CDRs were linked to the FRs from these human consensus sequences. The preliminary designs were examined and certain amino acid residues in the human FRs were identified as possible key residues in determining binding to antigen. For example, the amino acid residues that were part of the canonical structures for CDR loop formation, as proposed by Chothia *et al.* (1989), were highlighted (see residues marked with an asterisk in Figures 2 and 3). Residues that were potentially involved in V_L - V_H packing, as described by Chothia *et al.*

(1985), were examined. The rare occurrence of certain amino acids at specific positions was noted. With this information, and with reference to the model of the mouse C21 variable regions, decisions were made as to whether or not certain amino acid residues in the selected human FRs should be replaced with the amino acid residues that occurred at those positions in the mouse C21 variable regions.

For the design of the first version of reshaped human C21 V_L region (L1), changes in the human FRs were made at positions 1, 3, 49 and 60 (numbering according to Kabat *et al.*, 1987) (Figure 2). The amino acids at positions 1 and 3 were considered important because the model showed that the N-terminus of the mouse C21 light chain lay between CDR1 and CDR3 of the V_L region. Therefore, the N-terminus either could be directly involved in antigen binding or could alter the conformation of the CDRs. The amino acid at position 49 is lysine and is located between two amino acids that are part of the canonical structure for CDR2 of the V_L region. It is in the binding pocket created by CDR2 of the V_L region and may form an interaction with the glutamic acid at position 53 in CDR2 of the V_L region. The serine at position 60 in mouse C21 V_L region was located in the model at the edge of the binding site and could be influencing

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