different paths, and by ensuring that an azimuthally uniform coverage of stations is used in the averaging calculation. To compensate for other factors, such as focal depth, fault geometry and corner frequency would require such a detailed knowledge of the earthquake source that the M_s measurement itself would be redundant.

The results of this analysis can be summarized in five points.

(1) A global average moment-magnitude relationship $\bar{M}_s$ has been defined which can be used to predict M_0 over a wide range of magnitudes and scalar moments.

(2) The variance of surface wave measurements for an event of a particular scalar moment is ~0.2 magnitude units.

(3) Large regional biases in M_s exist.

(4) Differences in source scaling may explain some of the differences. Specifically, observations show that the transition from a slope of unity to a smaller value occurs at large moments for continental events than for ridge and fracture zone events, suggesting systematic differences in stress drop.

(5) Other systematic factors affecting the calculation of M_s also appear to contribute to the observed regional bias.

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Reshaping human antibodies for therapy

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A human IgG1 antibody has been reshaped for serotherapy in humans by introducing the six hypervariable regions from the heavy- and light-chain variable domains of a rat antibody directed against human lymphocytes. The reshaped human antibody is as effective as the rat antibody in complement and is more effective in cell-mediated lysis of human lymphocytes.

In 1890 it was shown that resistance to diphtheria toxin could be transferred from one animal to another by the transfer of serum. It was concluded that the immune serum contained an anti-toxin, later called an antibody[1]. For many years animal antisera were used in the treatment of microbial infections and for the neutralization of toxins in man[2]. More recently rodent monoclonal antibodies (mAbs)[3] have been used as 'magic bullets'[4] to kill and to image tumours[5,6]. The foreign immunoglobulin, however, can elicit an anti-globulin response which may interefere with therapy[7] or cause allergic or immune complex hypersensitivity[2]. Thus ideally human antibodies would be used. Human immunoglobulins are widely used as both prophylactic and microbicidal agents[8], but it would be far better to have available human mAbs of the desired specificity. It has proven difficult, however, to make such mAbs by the conventional route of immortalization of human antibody-producing cells[9].

There is an alternative approach. Antibody genes have been transfected into lymphoid cells, and the encoded antibodies expressed and secreted; by shuffling genomic exons, simple chimaeric antibodies with mouse variable regions and human constant regions have been made[10–12]. Such chimaeric antibodies have at least two advantages over mouse antibodies. First, the effector functions can be selected or tailored as desired. For example, of the human IgG isotypes, IgG1 and IgG3 appear to be the most effective for complement and cell-mediated lysis[13–15], and therefore for killing tumour cells. Second, the use of human rather than mouse isotypes should minimize the anti-globulin responses during therapy[16,17] by avoiding anti-isotypic antibodies. The extent to which anti-idiotypic responses to rodent antibodies in therapy are dictated by foreign components of the variable versus the constant region is not known, but the use of human isotypes should reduce the anti-idiotypic response. For example, when mice were made tolerant to rat immunoglobulin constant-region determinants, administration of rat anti-lymphocyte antibodies did evoke anti-idiotypic responses, but these were delayed and weaker than in animals that had not been made tolerant[18]. Nevertheless, it is likely that a chimaeric antibody would provoke a greater immune response than a human mAb.

We have attempted to build rodent antigen binding sites directly into human antibodies by transplanting only the antigen binding site, rather than the entire variable domain, from a rodent antibody. The antigen binding site is essentially encoded by the hypervariable loops at one end of the β-sheet framework. The hypervariable regions of the heavy chain of mouse antibodies against a hapten[19] or a protein antigen[47] were previously

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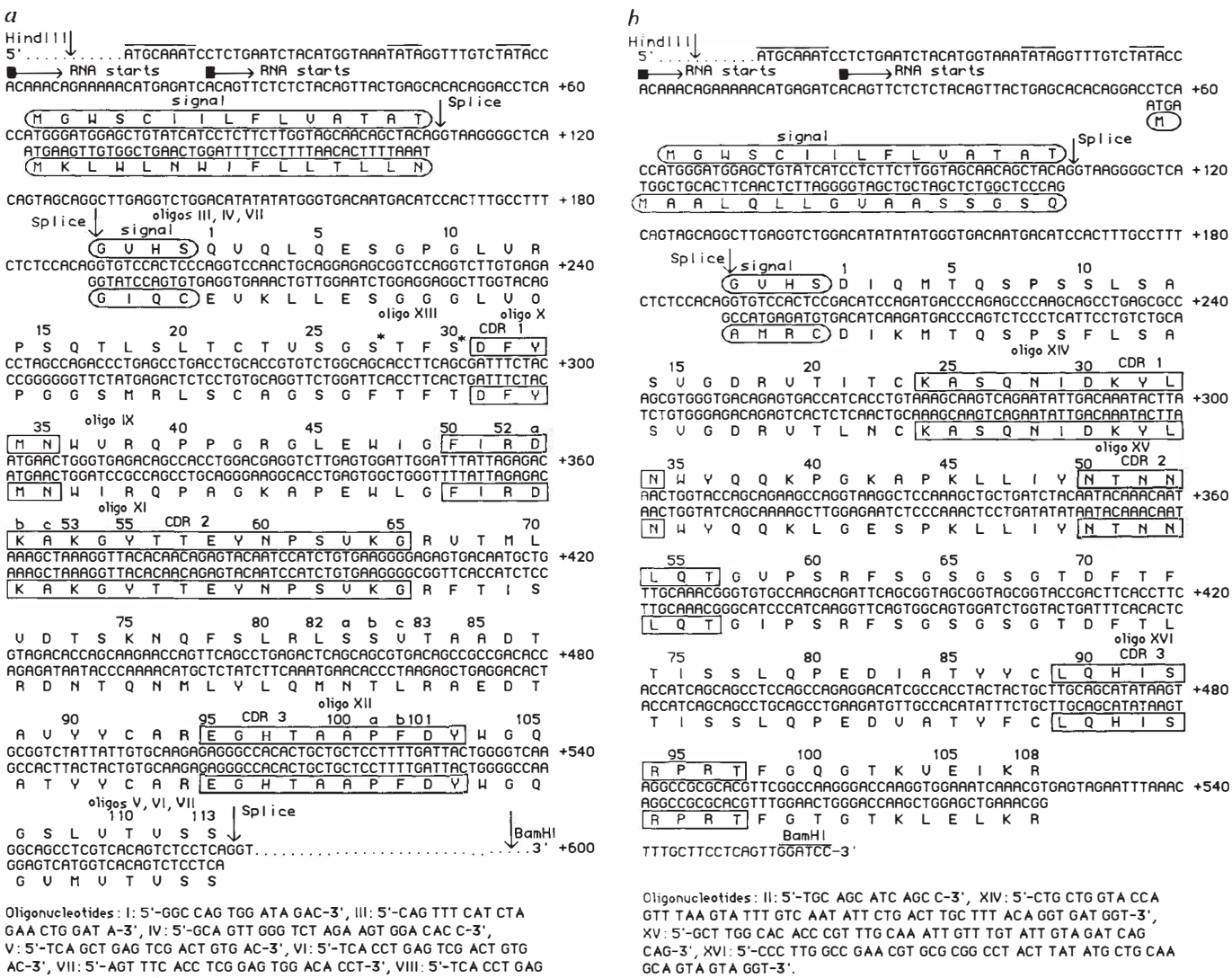

Fig. 1 Heavy-chain (*a*) and light-chain (*b*) sequences of the variable domains of reshaped (upper line) or rat YTH 34.5HL (lower line) antibodies. The reshaped heavy-chain variable domain HuVHCAMP was based on the HuVHNP gene[12,19], with the framework regions of human NEW (see note) alternating with the hypervariable regions of rat YTH 34.5HL. The reshaped light-chain variable domain HuVLCAMP is a similar construct, except with the framework regions of the human myeloma protein REI, with the C-terminal and the 3′ non-coding sequence taken from a human J_κ-region sequence[36]. The sequences of oligonucleotide primers are given and their locations on the genes are marked.

Methods. Messenger mRNA was purified[37] from the hybridoma clone YTH 34.5HL (γ2a, κ^b). First strand cDNA was synthesized by priming with oligonucleotides complementary to the 5′ end of the CH1 (oligonucleotide I) and the Cκ exons (oligonucleotide II), and then cloned and sequenced as described previously[38,39]. Two restriction sites (*Xba*I and *Sal*I) were introduced at each end of the rat heavy-chain variable region RaVHCAMP cDNA clone in M13 using mutagenic oligonucleotides III and V respectively, and the *Xba*I-*Sal*I fragment was excised. The corresponding sites were introduced into the M13-HuVHNP gene using oligonucleotides IV and VI, and the region between the sites was then exchanged. The sequence at the junctions was corrected with oligonucleotides VII and VIII, and an internal *Bam* HI site removed using the oligonucleotide IX, to create the M13-RaVHCAMP gene. The encoded sequence of the mature domain is thus identical to that of YTH 34.5HL. The reshaped heavy-chain variable domain (HuVHCAMP) was constructed in an M13 vector by priming with three long oligonucleotides simultaneously on the single strand containing the M13-HuVHNP gene[12,19]. Each oligonucleotide (X, XI and XII) was designed to replace each of the hypervariable regions with the corresponding region from the heavy chain of the YTH 34.5HL antibody. Colony blots were probed initially with the oligonucleotide X and hybridization positives were sequenced: the overall yield of the triple mutant was 5%. The (Ser27 → Phe) and (Ser27 → Phe, Ser30 → Thr) mutants of M13mp8-HuVHCAMP were made with the mixed oligonucleotide XIII. The reshaped light-chain variable domain (HuVLCAMP) was constructed in M13 from a gene with framework regions based on human REI (J. Foote, unpublished data). As above, three long oligonucleotides (XIV, XV and XVI) were used to introduce the hypervariable regions of the YTH 34.5HL light chain.

Note: There are discrepancies involving the first framework region and the first hypervariable loop of the NEW heavy chain between the published sequence[27] used here and the sequence deposited in the Brookhaven data base (in parentheses): Ser27 (→Thr), Thr28 (→Ser) and Ser30 (→Asp). Neither version is definitive (R. J. Poljak, personal communication) and the discrepancies do not affect our interpretations.

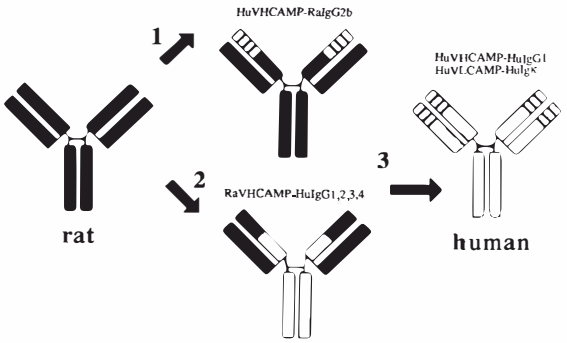

Fig. 2 Strategy for reshaping a human antibody for therapy. Sequences of rat origin are marked in black, and those of human origin in white. The recombinant heavy and light chains are also marked using a systematic nomenclature. See text for description of stages 1, 2 and 3. The genes encoding the variable domains were excised from the M13 vectors as *Hin*dIII-*Bam*HI fragments, and recloned into pSV2gpt[29] (heavy chains) or pSV2neo[30] (light chains), expression vectors containing the immunoglobulin enhancer[12]. The human γ1 (ref. 40), γ2 (ref. 41), γ3 (ref. 42), γ4 (ref. 41) and κ (ref. 36) and the rat γ2b (ref. 43) constant domains were introduced as *Bam*HI fragments. The following plasmids were constructed and transfected into lymphoid cell lines by electroporation[44]. In stage 1, the pSVgpt plasmids HuVHCAMP-RaIgG2B, HuVHCAMP(Ser→Phe)-RaIgG2B, HuVHCAMP-(Ser27→Phe, Ser30→Thr)-RaIgG2B were introduced into the heavy chain loss variant of YTH 34.5HL. In stage 2, the pSVgpt plasmids RaVHCAMP-RaIgG2B, RaVHCAMP-HuIgG1, RaVHCAMP-HuIgG2, RaVHCAMP-HuIgG3, RaVHCAMP-HuIgG4 were transfected as above. In stage 3, the pSV-gpt plasmid Hu(Ser27→Phe, Ser30→Thr)VHCAMP-HuIgG1 was co-transfected with the pSV-neo plasmid HuVLCAMP-HuIgK into the rat myeloma cell line Y0 (Y B2/3.0 Ag 20 (ref. 31). In each of the three stages, clones resistant to mycophenolic acid were selected and screened for antibody production by ELISA assays. Clones secreting antibody were subcloned by limiting dilution (for Y0) or the soft agar method (for the loss variant) and assayed again before 1 litre growth in roller bottles.

Since, to a first approximation, the sequences of hypervariable regions do not contain characteristic rodent or human motifs, such 'reshaped' antibodies should be indistinguishable in sequence from human antibodies.

There are mAbs to many cell-type-specific differentiation antigens, but only a few have therapeutic potential. Of particular interest is a group of rat mAbs directed against an antigen, the 'CAMPATH-1' antigen, which is strongly expressed on virtually all human lymphocytes and monocytes, but is absent from other blood cells including the haemopoietic stem cells[20]. The CAMPATH-1 series contains rat mAb of IgM, IgG2a and IgG2c isotypes[21], and more recently IgG1 and IgG2b isotypes which were isolated as class-switch variants from the IgG2a-secreting cell line YTH 34.5HL[22]. All of these antibodies, except the rat IgG2c isotype, are able to lyse human lymphocytes efficiently with human complement. Also the IgG2b antibody YTH 34.5HL-G2b, but not the other isotypes, is effective in antibody-dependent cell-mediated cytotoxicity (ADCC) with human effector cells[22]. These rat mAbs have important applications in problems of immunosuppression: for example control of graft-versus-host disease in bone-marrow transplantation[20]; the management of organ rejection[23]; the prevention of marrow rejection; and the treatment of various lymphoid malignancies (ref. 24 and M. J. Dyer, Hale, G., Hayhoe, F. G. J. and Waldmann, H., unpublished observations). The IgG2b antibody YTH 34.5HL-G2b seems to be the most effective at depleting lymphocytes *in vivo* but the use of all of these antibodies is limited by the anti-globulin response which can occur within

Table 1 Reshaping the heavy-chain variable domain

Heavy chain variable domain	Concentration of antibody in μg ml^{-1} at 50% antigen binding	50% complement lysis
RaVHCAMP	0.7	2.1
HuVHCAMP	27.3	*
HuVHCAMP (Ser27→Phe)	1.8	16.3
HuVHCAMP (Ser 27→Phe, Ser 30→Thr)	2.0	17.6

Antibodies with the heavy-chain variable domains listed above, rat IgG2b constant domains and rat light chains were collected from supernatants of cells at stationary phase and concentrated by precipitation with ammonium sulphate, followed by ion exchange chromatography on a Pharmacia MonoQ column. The yields of antibody were measured by an enzyme-linked immunosorbent assay (ELISA) directed against the rat IgG2b isotype, and each was adjusted to the same concentration[35]. To measuring binding to antigen, partially purified CAMPATH-1 antigen was coated onto microtitre wells and bound antibody was detected via a biotin-labelled anti-rat IgG2b mAb[35], developed with a streptavidin-peroxidase conjugate (Amersham). Complement lysis of human lymphocytes was with human serum as the complement source[21]. For both binding and complement assays, antibody titres were determined by fitting the data to a sigmoid curve by at least squares iterative procedure[21].

* Complement lysis with the HuVHCAMP variable domain was too weak for the estimation of lytic titre.

CAMPATH-1 antigen and the selection of human effector functions to match the lytic potential of the rat IgG2b isotype.

Strategy

The amino-acid sequences of the heavy- and light-chain variable domains of the rat IgG2a CAMPATH-1 antibody YTH 34.5HL were determined from the cloned complementary DNA (Fig. 1), and the hypervariable regions were identified according to Kabat[25]. In the heavy-chain variable domain there is an unusual feature in the framework region. In most known heavy-chain sequences Pro41 and Leu45 are highly conserved: Pro41 helps turn a loop distant from the antigen binding site and Leu45 is in the β bulge which forms part of the conserved packing between heavy- and light-chain variable domains[26]. In YTH 34.5HL these residues are replaced by Ala41 and Pro45 and presumably this could have some effect on the packing of the heavy- and light-chain variable domains. Working at the level of the gene and using three large mutagenic oligonucleotides for each variable domain, the rat hypervariable regions were mounted in a single step on the human heavy- or light-chain framework regions taken from the crystallographically solved proteins NEW[27] and REI[28] respectively (Fig. 1). The REI light chain was used because there is a deletion at the beginning of the third framework region in NEW. The reshaped human heavy- and light-chain variable domains were then assembled with constant domains in three stage (Fig. 2). This permits a step-wise check on the reshaping of the heavy-chain variable domain (stage 1), the selection of the human isotype (stage 2), and the reshaping of the light-chain variable domain and the assembly of human antibody (stage 3). The plasmid constructions were genomic, with the sequences encoding variable domains cloned as *Hin*dIII-*Bam*HI fragments and those encoding the constant domains as *Bam*HI-*Bam*HI fragments in either pSVgpt (heavy chain)[29] or pSVneo (light chain)[30] vectors. The heavy-chain enhancer sequence was included on the 5′ side of the variable domain, and expression of both light and heavy chains was driven from the heavy-chain promoter and the heavy-chain signal sequence.

Heavy-chain variable domain

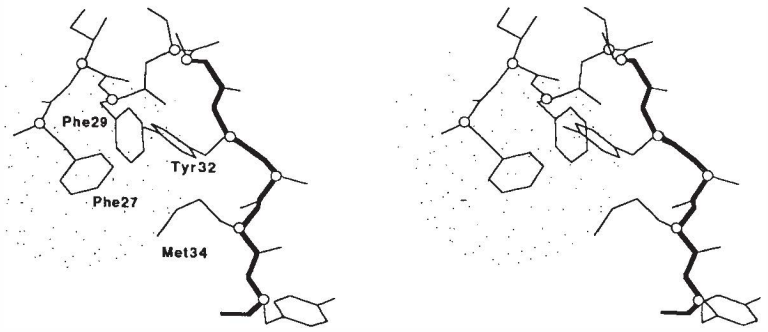

Fig. 3 Loop Phe27 to Tyr35 in the heavy-chain variable domain of the human myeloma protein KOL, which has been solved crystallographically[45]. The backbone of the hypervariable region according to Kabat[25] is highlighted, and a 200% van der Waal surface is thrown around Phe 27 to show the interactions with Tyr 32 and Met 34 of the Kabat hypervariable region. In the rat YTH 34.5HL heavy chain, these three side chains are conserved in character, but in HuVHCAMP, Phe27 is replaced by Ser.

isotype IgG2b and transfected into a heavy-chain loss variant of the YTH 34.5 hybridoma. This variant carries two light chains, one derived from the Y3 fusion partner[31]. The cloned rat heavy-chain variable domain (RaVHCAMP) was also expressed as above, and the antibodies were purified and quantified (Table 1). The HuVHCAMP and RaVHCAMP antibodies, each of the rat IgG2b isotype, were compared to the CAMPATH-1 antigen in a direct binding assay and in complement lysis of human lymphocytes (Table 1). Compared with the original rat antibody, or the engineered equivalent, the antibody with the reshaped heavy-chain domain bound poorly to the CAMPATH-1 antigen and was weakly lytic. This suggested an error in the design of the reshaped domain.

There are several assumptions underlying the transfer of hypervariable loops from one antibody to another[47], in particular the assumption that the antigen binds mainly to the hypervariable regions. These are defined as regions of sequence[25] or structural[32] hypervariability, the locations of hypervariable regions being similar by both criteria except for the first hypervariable loop of the heavy chain. By sequence the first hypervariable loop extends from residues 31–35 (ref. 25) whereas by structure it extends from residues 26–32 (ref. 32). Residues 29 and 30 form part of the surface loop, and residue 27, which is phenylalanine or tyrosine in most sequences, including YTH 35.5HL, helps pack against residues 32 and 34 (Fig. 3). Unlike most human heavy chains, in NEW (see note in Fig. 1) the phenylalanine is replaced by serine, which would be unable to pack in the same way. To restore the packing of the loop, we made both a Ser 27 → Phe mutation, and a Ser 27 → Phe, Ser 30 → Thr double mutation in HuVHCAMP. These two mutants showed a significant increase in binding to CAMPATH-1 antigen and lysed human lymphocytes with human complement (Table 1). Thus the affinity of the reshaped antibody could be restored by a single Ser 27 → Phe mutation, possibly as a consequence of an altered packing between the hypervariable regions and the framework. This suggests that alterations in the 'Kabat' framework region can enhance the affinity of the antibody and extends previous work in which an engineered change in the hypervariable region yielded an antibody with increased affinity[33].

Heavy-chain constant domains

In stage 2 (Fig. 2), the rat heavy-chain variable domain was attached to constant domains of the human isotypes IgG1, 2, 3 and 4, and transfected into the heavy-chain loss variant of the YTH 34.5 hybridoma. In complement lysis (Fig. 4*a*), the human IgG1 isotype proved similar to the YTH 34.5HL-G2b, with the human IgG3 isotype being less effective. The human IgG2 isotype was only weakly lytic and the IgG4 isotype was non-lytic. In ADCC (Fig. 4*b*) the human IgG1 was more lytic than the YTH 34.5HL-G2b antibody. The decrease in lysis at higher concentrations of the rat IgG2b and the human IgG1 antibody is due to an excess of antibody, which causes the lysis of effector

We therefore selected the human IgG1 isotype for the reshaped antibody. Other recent work also favours the use of IgG1 isotype for therapeutic application. When the effector functions of human isotypes were compared using a set of chimaeric antibodies with an anti-hapten variable domain, the IgG1 isotype appeared superior to the IgG3 in both complement and cell-mediated lysis[15]. Also, of two mouse chimaeric antibodies with human IgG1 or IgG3 isotypes directed against cell surface antigens as tumour cell markers, only the IgG1 isotype mediated complement lysis[13,14].

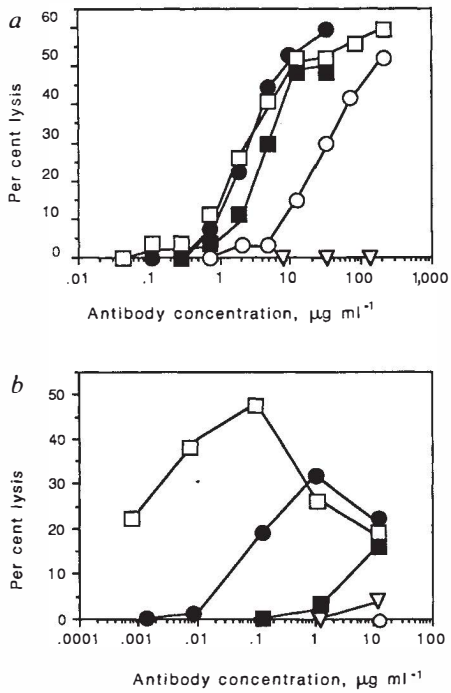

Fig. 4 *a*, Complement lysis and *b*, ADCC for antibodies with rat light-chain and rat heavy-chain variable domain attached to human IgG1 (□), IgG2 (○), IgG3 (■), or IgG4 (▽) isotypes. Lysis with the YTH 34.5HL antibody (●) is also shown.
Methods. Antibody was collected from cells in stationary phase, concentrated by precipitation with ammonium sulphate and desalted into phosphate buffered saline (PBS). Antibodies bound to the CAMPATH-1 antigen-coated on microtitre plates, were assayed in ELISA directed against the rat κ light chain[35], and each adjusted to the same concentration. The antibodies were assayed in complement lysis (Table 1) and ADCC with activated human peripheral blood mononuclear cells[35,46]. Briefly, 5×10^4 human peripheral blood cells were labelled with ^{51}Cr and incubated for 30 min at room temperature with different concentrations of antibody. Excess antibody was removed and a 20-fold excess of activated cells added

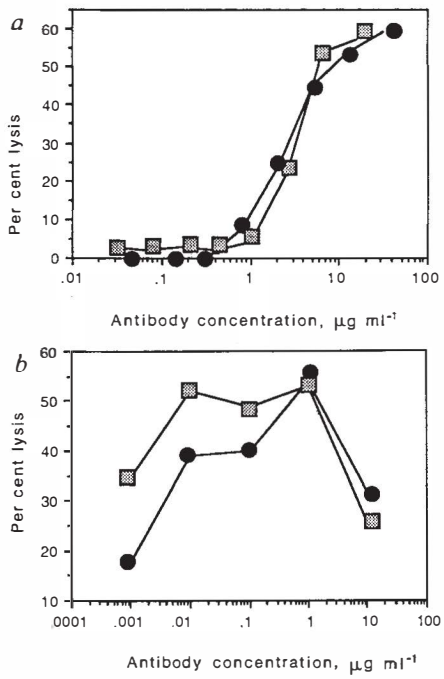

Fig. 5 *a*, Complement lysis and *b*, ADCC of the reshaped human (▩) and rat YTH 34.5HL (●) antibodies. Antibody HuVHCAMP (Ser27→Phe, Thr30→Ser)-HuIGG1, HuVLCAMP-HuIGK was purified from supernatants of cells in stationary phase by affinity chromotography on protein-A Sepharose. The yield (about 10 mg l^{-1}) was measured spectrophotometrically. Complement and ADCC assays were performed as in Fig. 4.

Light chain

In stage 3 (Fig. 2), the reshaped heavy chain was completed by attaching the reshaped HuVHCAMP (Ser27→Phe, Ser30→Thr) domain to the human IgG1 isotype. The reshaped light-chain domain HuVLCAMP was attached to the human Cκ domain. The two clones were co-transfected into the non-secreting rat Y0 myeloma line. The resultant antibody, bound to CAMPATH-1 antigen (data not shown), and proved almost identical to the YTH 34.5HL-G2b antibody in complement lysis (Fig. 5*a*). In cell-mediated lysis the reshaped human antibody was more effective than the rat antibody (Fig. 5*b*). Similar results were obtained with three different donors of target and effector cells (data not shown). Also, the antibody was as effective as YTH 34.5HL-G2b in killing leukaemic cells from three patients with B-cell lymphocytic leukaemia by complement-mediated lysis with human serum. Thus, by transplanting the hypervariable regions from a rodent to a human antibody of the IgG1 subtype, we have reshaped the antibody for therapeutic application.

Prospects

The availability of a reshaped human antibody with specificity for the CAMPATH-1 antigen should permit a full analysis of the *in vivo* potency and immunogenicity of an anti-lymphocyte antibody with wide therapeutic potential. Even if anti-idiotypic responses are eventually observed, considerable therapeutic benefit could be derived from an extended course of treatment. Also, it should be possible to circumvent an anti-globulin response restricted to idiotype by using a series of antibodies with different idiotypes[34]. In principle, the idiotype of the reshaped CAMPATH-1 could be changed by altering the hypervariable regions or the framework regions—evidence from a reshaped antibody specific for the hapten nitrophenyl acetate suggests that recognition by anti-idiotypic antisera and anti-idiotypic mAbs is influenced by residues in the framework region[19]. Thus, recycling the hypervariable regions on different human framework regions should change the idiotype, although ultimately it might focus the response directly onto the binding site for the CAMPATH-1 antigen. Although such focusing would be undesirable for CAMPATH-1 antibodies, it could be an advantage for the development of anti-idiotypic vaccines. It is likely that the answers to some of these questions will emerge from the use of this reshaped antibody in therapy.

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