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RECOGNITION OF QUEEN BEES

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RECOGNITION OF QUEEN-DERIVED

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A retinue of worker honey bees in the court of a glass pseudo-queen (in centre of photo) treated with a five-component synthetic queen mandibular gland blend. The blend initiates retinue formation, recognized here by the body alignment of the workers, head towards the stimulus. See page 354. And how bees see the third dimension, page 356.

THIS WEEK

Serotherapy hope

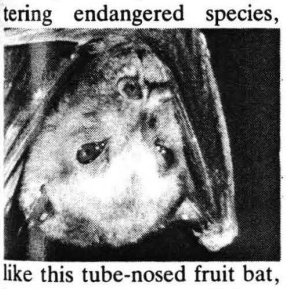
By engineering the hypervariable regions of a rat antibody raised against a human antigen into a human antibody sequence, it is possible to produce an antibody specific for human lymphocytes and with potential applications in serotherapy, page 323.

Proteins in stereo

An improvement in resolution means that three-dimensional NMR spectroscopy can now tackle the structures of macromolecules such as a 46-residue protein, pages 374 and 303.

Out of register

Is the present system for registering endangered species,



like this tube-nosed fruit bat, inadequate? See page 304.

Risk of quakes

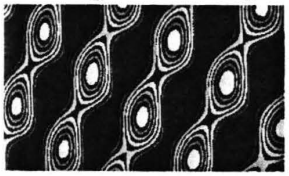
The traditional methods of measuring the magnitudes of earthquakes are of only limited value, so some estimates of seismic risk are suspect. New more rigorous scales are now needed, page 319.

Tin in the air

Decaying algal material in naturally reducing environments like salt marshes may volatilize tin as stannane — and other metals might be lost to atmosphere via similar routes, pages 339 and 309.

Microscopy by force

This colour molecular-resolution image shows the surface of a leucine crystal, as seen by the atomic force microscope, now being used on biological



molecules. White spots are highspots sensed by the atomic force probe, page 332.

Oven-to-table

A domestic microwave oven cooks up superconducting material, page 311. More on the latest cuprate superconductors, pages 334 and 305. And thin film technology, page 295.

Tree ring dating

Oak trees from bogs in Northern Ireland provide an accurate date for the violent volcanic eruption of the Aegean island Santorini, page 344.

Author Index

The Author Index for January and February, facing page 380.

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OPINION

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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 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 IgGI 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 transplanted into a human heavy chain, and, in association with the mouse light chain, the antigen binding site was retained.

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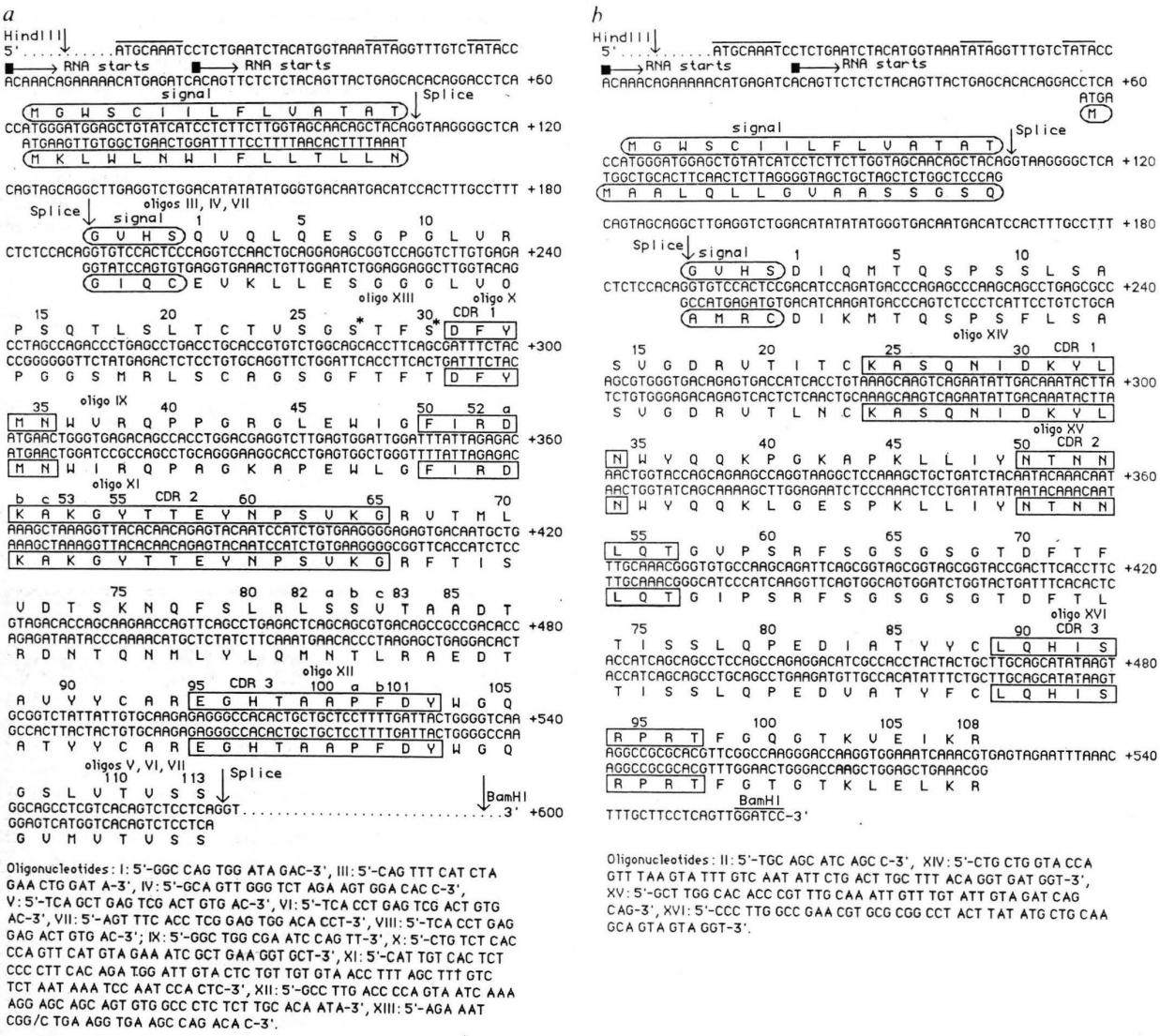

Fig. 1 Heavy-chain (*a*) and light-chain (*b*) sequences of the variable domains of reshaped (upper line) or rat YTH 34.5HL (lower line) antibodies. The reshaped heavy-chain variable domain HuVHCAMP was based on the HuVHNP gene[12,19], with the framework regions of human NEW (see note) alternating with 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.

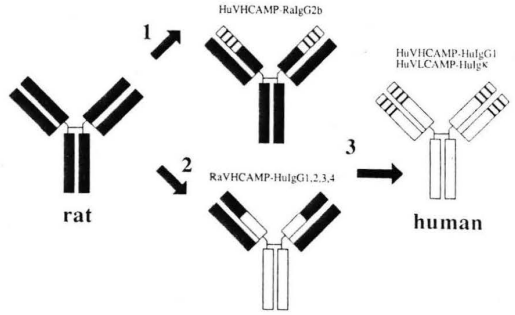

Fig. 2 Strategy for reshaping a human antibody for therapy. Sequences of rat origin are marked in black, and those of human origin in white. The recombinant heavy and light chains are also marked using a systematic nomenclature. See text for description of stages 1, 2 and 3. The genes encoding the variable 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 two weeks of the initiation of treatment[24]. Here we describe the reshaping of human heavy and light chains towards binding the CAMPATH-1 antigen and the selection of human effector functions to match the lytic potential of the rat IgG2b isotype.

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.

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

In stage 1, the reshaped heavy-chain variable domain (HuVHCAMP) was attached to constant domains of the rat

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