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Engineering Antibodies for Therapy

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Introduction

Antibodies have long been viewed as potential agents for targeted drug delivery and other therapeutic interventions, largely with a view to exploiting the combination of high specificity and affinity of the antibody–antigen interaction. Since the development of rodent monoclonal antibody (MAb) technology (Kohler and Milstein, 1975) it has been possible in principle to produce rodent MAbs to virtually any antigen, and a large number of rodent MAbs relevant to human therapy have been generated. MAbs have already been used clinically for the diagnosis and therapy of several human disorders, notably cancer and infectious diseases, and for the modulation of immune responses. The target antigens have been tumour-associated antigens (TAAs, Boyer *et al.*, 1988; Herlyn, Menrad and Koprowski, 1990), specific cell type markers, viruses, bacteria and specific human proteins of physiological

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; bp, base pairs; BSA, bovine serum albumin; c[antibody name], mouse variable region–human constant region chimeric[antibody name]; ADCMC, antibody-dependent complement-mediated cytotoxicity; cDNA, complementary DNA; CDR, complementarity determining regions; CEA, carcinoembryonic antigen; CTL, cytotoxic T lymphocyte; d, days; DHFR, dihydrofolate reductase; DNS, dansyl; ELISA, enzyme-linked immunosorbent assay; E:T, effector-to-target ratio; FITC, fluorescein isothiocyanate; GM-CSF, granulocyte-macrophage-colony-stimulating factor; *gpt*, xanthine/guanine phosphoribosyl transferase gene; h, hours; HAMA, human anti-mouse antibody; HbsAg, hepatitis B surface antigen; hCMV, human cytomegalovirus; hEGFR, human epidermal growth factor receptor; HIV, human immunodeficiency virus; *hph*, hygromycin B phosphotransferase gene; HRP, horse radish peroxidase; hyg^r, hygromycin resistance; IC_n, quantity required for n% inhibition of activity; ID_n, quantity of virus required for n% infection; IFNγ, interferon γ; i.n., intra-nasal; i.p., intra-peritoneal; i.v., intra-venous; kbp, kilobase pairs; KLH, keyhold limpet haemocyanin; LT, lymphotoxin; LTR, long terminal repeat unit; MAb, monoclonal antibody; MLR, mixed lymphocyte reaction; mRNA, messenger RNA; MSX, methionine sulphoximine; MTX, methotrexate; *neo*, neomycin phosphotransferase; NIP, 5-iodo-4-hydroxy-3-nitrophenacetyl; NP, 4-hydroxy-3-nitrophenacetyl; NP-cap, NP-caproic acid; OD_n, optical density at n nm; % $i.d.g^{-1}$, percentage of injected dose per gram of tissue; *o*-PDM, *N,N'*-1,2-phenylenedimaleimide; PBMC, peripheral blood mononuclear cells; PC, phosphorylcholine; PEG, polyethylene glycol; PGK, phosphoglycerate kinase; pfu, plaque-forming units; PLAP, placental alkaline phosphatase; PMN, polymorphonuclear lymphocyte; p/o, promoter/operator; rbs, ribosome binding site; RES, reticulo-endothelial system; rIL-2, recombinant interleukin 2; s.c., subcutaneous; SDM, site-directed mutagenesis; SRBC; sheep red blood cells; TAA, tumour-associated antigen; TNB, thionitrobenzoate; TNP, trinitrophenyl; V_H, heavy chain variable domain; V_L, light chain variable domain.

importance (particularly cytokines or their receptors). At the end of 1991 there were 132 biotechnology-based medicines in formal clinical development (Pharmaceutical Manufacturers Association Report, 1992) or awaiting final approval from the US Food and Drugs Administration (FDA). Sixty-six of the 132 were for cancer therapy and 58 of the 132 were MAbs. Three rodent MAbs have so far been approved and launched as products: OKT3 is a naked MAb and has been approved by the FDA for treatment of acute kidney transplant rejection; OncoScint and MyoScint are Mab–isotope conjugates which have been approved outside the US as imaging agents for colorectal cancer and myocardial infarction, respectively. The human MAb 'Centoxin' has also been approved in Europe for treatment of septic shock. The total market for MAbs is presently around $330 million per annum and is estimated to grow approximately ten-fold by 1996. Therapeutic MAbs will account for most of this market.

Although the specificity of MAbs undoubtedly gives them immense potential in medicine, rodent MAbs are certainly not ideal therapeutic agents. The five most important issues and technical challenges in the development of MAb-based therapies are: (1) identifying MAbs of suitable affinity and specificity; (2) overcoming human immune responses against rodent MAbs and against any cell-killing agents attached to them; (3) identifying and harnessing appropriate cell-killing agents; (4) achieving appropriate pharmacokinetics and biodistribution; (5) achieving economic manufacture, which is of particular relevance for highly engineered MAbs and for MAb–cytotoxic agent conjugates (as opposed to naked MAbs).

As the above statistics indicate, a large proportion of the MAb-based agents presently in clinical development are for treatment of cancer and in this review the development of anti-cancer MAbs and MAb conjugates will largely be used to illustrate the approaches being taken to address the five key issues. The review begins with a brief description of the structure of antibodies and antibody genes, followed by a summary of the arguments and evidence relating to the importance of affinity and specificity for MAb-based therapies. We then briefly summarize the available clinical results with naked rodent MAbs. Next we describe the approaches being taken to overcome the immunogenicity in patients of rodent MAbs, which is certainly the most serious and general problem for MAb-based therapies. This section concentrates largely on antibody humanization, which is the most promising solution to the problem. The processes developed for efficient cloning of antibody genes and for production of engineered whole antibodies are then described. This is followed by a summary of the approaches being taken to improve the pharmacokinetics and biodistribution of MAbs, focusing particularly on the development of engineered antibody fragments, and then by a summary of production systems being used for such fragments. The first half of the review is then completed by a summary of the various cell-killing strategies being developed for MAb-based therapies. The second half of the review is largely devoted to a detailed summary of the construction, expression, pre-clinical

The review finishes with some conclusions and a summary of recent advances in antibody technology which may lead eventually to the successors of humanized rodent MAbs for therapy.

The structure of antibodies and antibody genes

In order to understand the later sections of this review (especially those on antibody gene cloning and humanization) it is necessary to have some knowledge of the structure and organization of antibodies and their genes. We give a brief description here.

Higher mammals have five classes of immunoglobulin, termed IgG, IgM, IgA, IgE and IgD. The structures and functions of these five classes have been very well described by Roitt, Brostoff and Male (1987). Almost all MAbs of therapeutic potential are of the IgG class, and have the basic structure shown in *Figure 1A*. IgG antibodies have a tetrameric structure consisting of two identical 55 kDa glycosylated proteins (termed 'heavy chains') and two identical 25 kDa proteins, which are normally not glycosylated (termed 'light chains'), covalently linked by disulphide bridges. The proteins are organized into discrete folding domains of around 110 amino acids which are encoded in the genome on separate exons (*Figures 1B and 1C*). Each light chain associates with and is covalently linked via a disulphide bridge to a cysteine in the N-terminal region of one heavy chain, and the C-terminal half of the heavy chains associate with each other to form a Y- or T-form structure. The heavy chains are also covalently linked to each other via disulphide bridges in the hinge domain.

Sequence information is now available for hundreds of antibodies of many different species and reveals that the N-terminal domains of each chain are much more variable in sequence than the other domains. The N-terminal domains are therefore termed 'variable domains' and the others 'constant domains'. Three non-contiguous regions within these variable domains are particularly variable and are usually referred to as 'hypervariable loops' or 'complementarity determining regions' (CDRs). This sequence variation is postulated to provide the variability (within these otherwise highly conserved proteins) which enables antibodies to recognize and bind to a very wide range of antigens (Wu and Kabat, 1970; Kabat *et al.*, 1987). The proposal has been confirmed by structural studies, which show that the hypervariable sequences are (in most cases) associated on the surface of the antibody as a set of loops. The loops form a large surface patch and are in contact with antigen in cases for which structural information on the antibody–antigen complex is available (Amit *et al.*, 1986; Boulot *et al.*, 1987; Colman *et al.*, 1987; Sheriff *et al.*, 1987; Davies *et al.*, 1989; Padlan *et al.*, 1989; Tulip *et al.*, 1989, reviewed in Alzari *et al.*, 1987; Bentley *et al.*, 1990; Bhat *et al.*, 1990; Davies, Padlan and Sheriff, 1990 and Poljak, 1991). The variable region residues that are not part of the CDR or loops together constitute the 'framework' of the variable region. It has been shown that the exons for the variable domains are assembled from a

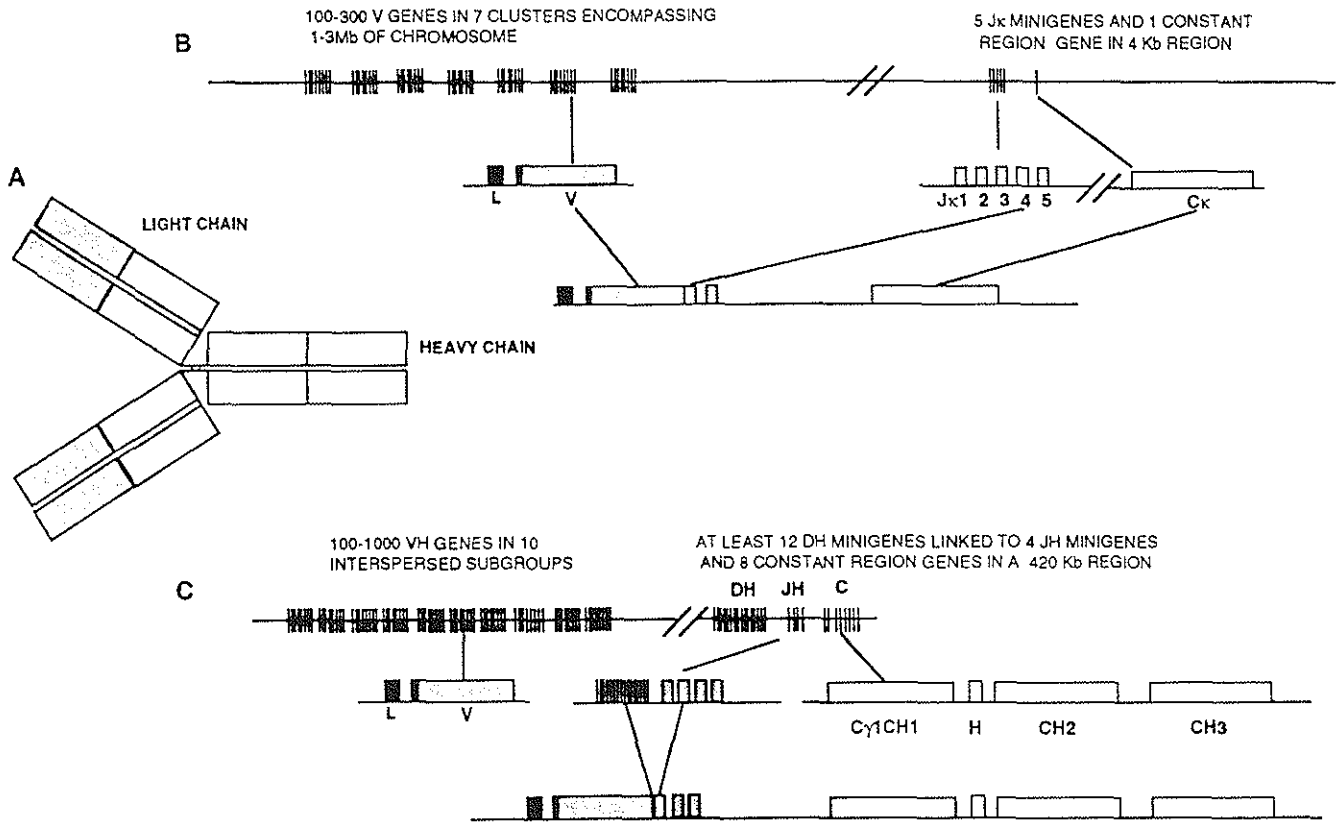

Figure 1. Protein and gene organization of murine IgGκ.

development of the antibody-producing B-cell lineage. The variable region exon along with the signal sequence exon and the promoter/enhancer (involved in transcription) is then juxtaposed with the constant region gene family by further recombination events for subsequent expression (*Figures 1A, 1B*; reviewed by Alt, Blackwell and Yancopolous, 1987). The organization of the Ig loci in mice and humans has been reviewed recently by Lai, Wilson and Hood (1989).

The constant regions tend to be conserved in sequence among antibodies of a given species, and also to a lesser extent between species. Light chains have a single constant domain for which there are two gene loci, Cκ and Cλ. IgGs have three constant domains on the heavy chains, CH1, CH2 and CH3. Between the CH1 and CH2 domains (for IgGs) is a short proline-rich peptide sequence termed the 'hinge' which contains the cysteines that bridge the two heavy chains. IgGs also have a site in the CH2 domain for *N*-linked glycosylation, which is required for structural integrity of the antibody and for some of its effector functions. Sequence motifs within the CH2 and CH3 domains are responsible for the effector functions, such as complement activation and binding to other cells of the immune system. In humans and rodents there are four different types of IgGs, termed 'isotypes', which vary in their spectrum of effector functions as a result of amino acid sequence variation in the constant regions (Burton, 1990). In humans there are a number of immunologically distinct variants of IgG1, 2 and 3, termed allotypes (Gorman and Clark, 1990). These allotypes are racially distributed, for example the G1m(3) marker predominates in Caucasian IgG1 whereas G1m(1,17) predominates in Asian and Japanese individuals.

Until the advent of recombinant DNA technology antibody fragments (*Figure 2*) were generated by proteolytic digestion. Pepsin cleaves IgGs on the C-terminal side of the hinge, liberating an antigen binding fragment referred to as the $F(ab')_2$. Papain cleaves on the N-terminal side of the hinge and liberates two F(ab) fragments and a single Fc fragment. The F(ab) fragments have a single antigen binding site (monovalent), while the $F(ab')_2$ has two (bivalent). The term F(ab′) means monovalent but with the hinge sequence also present. The heavy chain of the F(ab) or F(ab′) is usually referred to as the Fd or Fd′. The variable domains of the heavy and light chains (V_H and V_L) together comprise a fragment called the Fv. This is the smallest fragment which retains the full antigen binding activity of the monovalent antibody. Although the Fv can be obtained for some antibodies by proteolytic digestion of the IgG the process is very inefficient. Fvs dissociate into V_H and V_L under physiological conditions, and so are not useful for therapy. The single chain Fv (scFv) represents the most successful strategy for stabilizing the Fv. It has V_H and V_L linked by a short peptide linker (between the C-terminus of one domain and the N-terminus of the other) and expressed as a single polypeptide chain. It is possible to make scFv variants for most MAbs which retain most or all of the monovalent antigen binding activity of the MAb. In some cases V_H alone displays significant

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