Therapeutic Immunology

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Arnette Blackwell SA, 1 rue de Lille, 75007 Paris, France
Blackwell Wissenschafts-Verlag GmbH
Kurfürstendamm 57, 10707 Berlin, Germany
Feldgasse 13, A-1238 Vienna, Austria

Distributors:

North America
Blackwell Science, Inc.
238 Main Street
Cambridge, Massachusetts 02142
(Telephone orders: 800-215-1000 or 617-876-7000)

Australia
Blackwell Science Pty Ltd
54 University Street
Carlton, Victoria 3053
(Telephone orders: 03-347-0300
fax: 03-349-3016)

Outside North America and Australia
Blackwell Science, Ltd.
c/o Marston Book Services, Ltd.
P.O. Box 87
Oxford OX2 0DT
England
(Telephone orders: 44-1865-791155)

Acquisitions: Victoria Reeders
Development: Kathleen Broderick
Production: Tracey Solon
Manufacturing: Karen Feeney
Printed and bound by: Braun-Brumfield, Inc.

Printed in the United States of America
96 97 98 99 5 4 3 2 1

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Library of Congress Cataloging-in-Publication Data

Therapeutic immunology / [edited by] K. Frank Austen . . . [et al.].
p. cm.
Includes bibliographical references and index.
ISBN 0-86542-375-X (alk. paper)
1. Immunotherapy. I. Austen, K. Frank (Karl Frank)
[DNLM: 1. Immunotherapy. 2. Immune System—drug effects. QW 940 T398 1996]
RM275.T44 1996
615'.37—dc20
DNLM/DLC
for Library of Congress 95-51338
CIP

Production and Manipulation of Antibodies and T-Cell Receptors Using Recombinant DNA Technology

E. Sally Ward

The antigen receptors of B and T cells are responsible for mediating immune recognition and, therefore, play a critical role in defense against pathogens such as bacteria and viruses. The high specificity of these receptors makes them attractive for use as reagents in diagnosis and therapy (1). Understanding the antibody-antigen and T-cell receptor (TCR)–peptide–major histocompatibility complex (MHC) interactions at the molecular level is of central importance in immunology. For the production of large amounts of an antibody in clonal form, the development of hybridoma technology (2) followed by recombinant expression systems (3–5) has proven invaluable for analyses of antibody structure and interaction with cognate antigen. For TCRs, however, the invariably membrane-bound TCR has proven more difficult to produce as a soluble molecule, and only recently have efficient systems been described.

This review describes the recent developments in the field of antibody engineering using *Escherichia coli* as a host for expression. In addition to mammalian systems, *E. coli* systems have also been developed to produce soluble TCRs for use in structure-function studies and immunotherapy, and work in this area is briefly discussed.

THE ANTIBODY MOLECULE

The antibody molecule is composed of discrete domains that are linked together by relatively flexible peptides (6–11). The length of the hinge region is variable from one isotype to another, affecting the flexibility of the Fab arms relative to the Fc portion. Segmental flexibility has been shown to be important for gammaglobulins (immunoglobulin G, or IgG) to carry out complement fixation (12, 13), but more recently it has been demonstrated that complement-mediated cell lysis can be carried out by a hinge-deleted IgG (14). The immunoglobulin (Ig) domains are made up of antiparallel β sheets that are pinned together by an intramolecular disulphide bridge, and this structure is called the immunoglobulin fold. Proteins that are made up of Ig-like domains constitute the immunoglobulin superfamily (15) and usually differ in the number and length of the strands in the two β sheets and in the size and conformation of the loops that link the ends of the strands. These different members have been classified into groups called sets (15), of which there are currently 4 (16). For the variable domains, which are members of the V set and confer the antigen-binding specificity and affinity on the Ig molecule, the β strands support the hypervariable loops or complementarity-determining regions (CDRs). There are three CDRs per variable domain, and it is residues within and flanking these loops that are involved in interacting with antigen on antibody-antigen contact (17–21). The almost unlimited potential to generate variable region diversity by somatic recombination of V, (D) and J elements, imprecise joining of these segments, and N-addition (22) results in a V gene repertoire of enormous diversity in any one individual. Superimposed on this germline diversity is the process of affinity maturation (23, 24), which results in the selection, after somatic mutation, of B cell clones that produce antibodies of higher affinity from a pool of background lower affinity clones.

The antibody heavy- and light-chain variable (VH and VL, respectively) domains constitute the Fv frag-

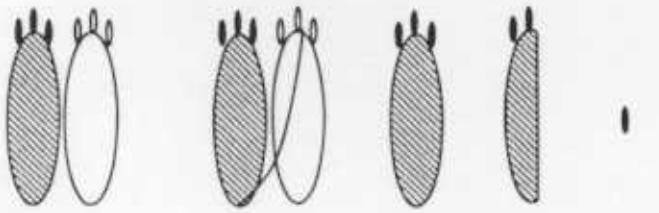

FIGURE 25.1 *The "minimal" units of antigen binding. Each immunoglobulin V domain is represented by large ellipsoids, with complementarity-determining region (CDR) loops represented by small ellipsoids. The single-chain linker peptide in the single-chain Fv (scFv) (84, 85) is represented by a single line. The minibody (32, 33), a 61-residue truncated VH domain has only two CDR loops (corresponding to CDR1 and 2). (MRU = minimal recognition unit) (30, 31).*

ment, which is conventionally regarded as the minimal unit of antigen binding (25, 26). However, VH domains (27–29) and CDR-derived peptides (30, 31) with reasonable affinities for cognate antigen have been described (Fig 25.1). Protein engineering has also been used to design and build a minibody composed of 61 amino acids using the Ig VH domain as a template (32, 33). This 61-residue all-β variable domain has a novel β sheet scaffold and hypervariable regions corresponding to CDR1 and 2. This scaffold may prove to be particularly useful for the de novo design of antigen-binding sites.

The constant regions of IgGs are involved in effector functions, for example, complement fixation and binding to Fc receptors to perform antibody-dependent cell-mediated cytotoxicity (ADCC) (34). Antibodies of different isotypes differ in their ability to mediate ADCC and fix complement (35, 36); therefore, genetic engineering can be used to perform isotype switching in vitro to produce an antibody with the desired effector functions (35). The serum persistence of the murine IgG1 molecule is controlled by sequences located at the C_H2-C_H3 domain interface (37), and this region overlaps with the binding site for Staphylococcal protein A (8). The identification of this region as being involved in the control of the pharmacokinetics of antibodies may allow the clearance rates of therapeutic IgGs to be manipulated. Another function of the constant region that is of value, particularly for lower affinity antibodies, is to hold the two Fab arms together, resulting in the generation of a higher avidity antibody.

EXPRESSION OF ANTIBODIES IN *ESCHERICHIA COLI*: THE BINDING SITE

Genetic manipulation of the antigen-binding site of antibodies has been greatly assisted by the development of prokaryotic expression systems that result in the secretion of either Fv or Fab fragments or single VH domains into the periplasmic space (27, 38, 39). Before this, attempts to express antibody fragments in *E. coli* resulted in limited success, although more recently a number of systems for intracellular expression, isolation of the antibody fragments, denaturation, and refolding have been reported (40). For secretion systems, the exported antibody fragments can be isolated from either the periplasm (38) or, as a result of leakage into the culture medium, from the supernatant (27). Culture supernatants can, therefore, be screened directly for the presence of binding activities. This avoids the need for lysis of the bacteria followed by refolding of the recombinant protein, which is clearly not attractive for the analysis of large numbers of clones.

USE OF THE POLYMERASE CHAIN REACTION FOR THE ISOLATION OF ANTIBODY V DOMAIN GENES

Antibody VH and VL domain genes can now be isolated from antibody-producing cells for a number of different species using the polymerase chain reaction (PCR) (41) and primers designed to anneal to the 5′ end of the VH/VL gene and JH/JL regions or C_H1/Cκ regions (27, 42–47). Primers that anneal to the secretion leader sequences can also be used (43, 45), but generally these are not as convenient for in-frame ligation of the isolated Ig genes into Fv or Fab expression plasmids. Using the primers, V genes can be isolated from hybridomas for expression and sequence analysis (42, 43, 45). A more challenging direction is to use the primers to generate diverse repertoires of Ig V genes that can be isolated in a single step (27, 46). These genes can then be used to generate libraries of antibody Fv or Fab fragments from which clones producing the desired antigen-binding specificity can be isolated by screening (27, 46) or, more recently, by selection using bacteriophage display (48–50).

Bacteriophage display

The extension of peptide display libraries (51) to the display of proteins on the surface of bacteriophage has resulted in systems (48–50, 52) in which Fv or Fab fragments can be rapidly isolated from diverse V gene libraries by selection. Before this, libraries were screened by either growing up recombinant clones and analyzing culture supernatants (27) or probing colony lifts with labeled antigens (46). Both methods suffer limitations in the numbers of clones that can be easily screened. As an alternative, phage display allows selection from library sizes of almost unlimited numbers that approach the size of the immune repertoire (53, 54). The limitation has now switched to the generation of large numbers of recombinant clones and the use of

the PCR to generate highly diverse libraries without biases toward particular Ig gene families. The former can be overcome, in part at least, by using electroporation to generate clones with extremely high transformation frequencies and the latter by the judicious choice of primers.

Isolation of antigen-binding specificities: the generation of antibodies for therapy

Using the methods just described, Fv and Fab fragments with binding specificities toward an array of hapten and protein antigens have been isolated from both mice and humans (53–62). Many of these antibodies are of therapeutic value and, for example, have neutralizing activity against viruses (58, 59, 62). The advantage of prior immunization is that it improves the chances of isolating high-affinity, somatically mutated antibodies. However, for humans, immunization is generally not possible for obvious reasons, and only in the cases of infected patients (e.g., HIV infected; 58, 60) or vaccinated people are "immunized" donors available. For use in therapy, it has become clear that antibodies of human origin are preferable because of the anti-Ig or anti-idiotypic response (4) that occurs after treatment with either rodent antibodies or simple chimeric antibodies (the latter composed of rodent V regions linked to human constant regions) (63, 64), respectively. In building antibodies for therapy, human constant regions of the appropriate allotype should be used to avoid antiallotypic responses (65). To generate high-affinity human antibodies for use in therapy, several approaches have been described. These involve the isolation of either Fv or Fab fragments, which can then be used to rebuild complete antibodies or the isolation of complete human antibodies in one step, as follows.

1. To immunize mice and isolate the binding specificity of choice from Ig gene libraries. The CDR loops (and if necessary, flanking regions) can then be grafted onto human V gene frameworks to produce a humanized antibody (66–72). However, this is usually not straightforward and to produce a humanized antibody that has the same affinity as the parent rodent antibody, it is frequently necessary to mutate flanking framework region residues (e.g., 67, 70). The effects of framework mutations on the affinity of an antilysozyme antibody has been analyzed in detail by Foote and Winter (73). Furthermore, for antigens that are not immunogenic (e.g., highly conserved cellular proteins), rodent antibodies cannot be raised and as a result there is no "donor" antibody available.

2. To isolate antibodies from human-derived "one-pot" libraries (61) and, if necessary, to improve the affinities by rounds of mutagenesis followed by selection. In this approach, large libraries (approximately 10^8 members) of V genes derived from humans are assembled as scFv fragments and displayed on the surface of phage. From these libraries, scFv fragments with reasonable affinities for binding to antigen can be isolated. The affinities can be further improved by using the scFv genes as templates for point mutagenesis (74, 75), insertion of random cassettes into regions corresponding to CDRs (76, 77) or chain shuffling (78–80).

3. To repopulate severe combined immunodeficiency mice with human peripheral blood lymphocytes (PBLs) (81). In this approach, the PBLs were immunized in vitro before injection into mice, and mice were also immunized after transfer of the PBLs. Fab fragments with specificities for hepatitis B core antigen and tetanus toxoid were isolated using recombinant methods (81).

4. Transgenic mice that have human Ig gene miniloci have been described (82, 83). The human genes are rearranged into a functionally active form, and immunization with human CD4 (82) or tetanus toxin (83) results in a normal response to antigen that involves both class switching and somatic mutation. These transgenic animals promise to provide a rich source of antibodies of human origin. Furthemore, bacteriophage display systems could be used in concert with these systems to rapidly isolate and express the binding specificities in clonal form.

In vitro somatic maturation using bacteriophage display

Diversity of V gene repertoires can be increased using the PCR to either insert random point mutations (74, 75) or synthetic CDRs (76, 77). The randomly mutated V genes can subsequently be expressed as scFvs or Fabs on the surface of bacteriophage and higher affinity variants selected by panning. Using PCR conditions designed to insert random point mutations the affinity of a murine anti-4-hydroxy-5-iodo-3-nitrophenacetyl-caproic acid antibody was increased 4-fold (74) and that of a murine antiprogesterone antibody 30-fold (75). The PCR mutagenesis can be followed by rounds of selection using conditions designed to select for antibodies with lower off rates (74). This approach has clear implications for the improvement of affinities of any antibody of interest but should be of particular utility in increasing the affinity of low-affinity human antibodies derived from "naive" libraries or for antibodies that recognize antigens of low immunogenicity.

To generate junctional diversity in vitro, PCR or cassette mutagenesis can be used to insert CDR3s (and other CDRs) of random sequences (76, 77) to generate semisynthetic V gene libraries. Again, this approach is of particular value for the generation of antibodies of

human origin and has to date been applied to isolate antibodies that recognize both protein and hapten antigens (76, 77).

Single-chain Fvs or Fabs?

The availability of cloning systems for bacteriophage display of either scFv or Fab fragments prompts the question as to whether it is preferable to express scFvs or Fabs using *E. coli* as a host. Originally, scFv fragments were designed to covalently link the VH and VL domains to avoid dissociation (84, 85). For some Fv fragments, covalent linkage has proven necessary, as the VH-VL association constant appears to vary widely from one Fv to the next. An alternative approach to using a single-chain linker is to insert cysteine residues at positions in the VH and VL domains that are predicted or known to be close to each other (86–88). The coexpression results in the production of −S-S–linked heterodimers, and these may be more resistant to thermal denaturation than scFv fragments (86). However, for cloning of Fv fragments as libraries on the surface of bacteriophage, it is clearly more straightforward to link the VH and VL domains by a single-chain linker. There are examples of Fv fragments for which the VH-VL association constant is relatively stable and, if this is the case, has advantages as unlinked Fv fragments may be expressed in much higher yields than the corresponding scFv fragments (80). The in vivo stability of these unlinked Fv fragments, however, is questionable, and because unlinked domains may dissociate and bind nonspecifically to other proteins (89) with undesirable side effects, covalently linked VH-VL dimers are preferable for use in therapy and diagnosis.

ScFV fragments have a tendency to dimerize or aggregate to form higher order multimers (54, 90, 91). Dimerization is in some cases an advantage because it increases the valency of the fragment (see later discussion). However, for high-resolution structural studies aggregation of scFv fragments can cause problems, although to date, the x-ray structures of several scFv fragments are underway or solved (91, 92). It has been suggested that steric strain of the linker causes this aggregation, which indicates that for some scFv fragments at least, lengthening the linker may alleviate this effect (91).

The expression of Fab fragments avoids the potential problems of scFv multimerization, but in some cases, deletion of light chains has been observed during selection of Fab-bearing bacteriophage (75) from repertoires. In addition, it has been shown that antibodies of high affinities can be made by renaturation of light chains with repertoires of heavy chains displayed on the surface of phage (93) or by recombination of diverse heavy- and light-chain repertoires in bacteria (94). Clearly, these powerful approaches for the isolation of high-affinity antibodies of human origin are not possible using scFv display libraries, and the use of Fab expression systems has obvious advantages. Thus, the choice between scFv and Fab fragments depends on the approach that will be used to generate the binding specificity; after isolation of fragments with the desired affinity and specificity, it is straightforward to use genetic manipulation to interconvert the two types of fragments.

Uses of scFv and Fab fragments in therapy: extension to bivalent and bispecific fragments

The rapid clearance rates of (sc)Fv and Fab fragments (89, 95) indicates that they are suitable for use as imaging reagents and in therapeutic situations in which the typically long half-life of a complete IgG molecule is not needed. For imaging, rapid clearance is an advantage because it reduces background. For higher avidity fragments, bivalent (sc)Fv and Fab fragments can be produced using a variety of recombinant approaches that can readily be extended to the production of bispecific Fab and (sc)Fv fragments by the following methodologies:

1. −S-S–linked dimers: An extra cysteine residue has been engineered into the C-termini of each of the C_H1 domains of two Fab fragments of different specificities (96). The Fab fragments were expressed separately, purified from the periplasm, and chemically coupled to each other in vitro. More recently, coexpression of bivalent Fab fragments with repeated Cys-Pro-Pro motifs (97) has been shown to result in the production of high yields of bispecific Fab fragments in *E. coli*. A similar approach has been used to produce bivalent Fv fragments (89).

2. Dimerization domains: scFv fragments have been extended at the C-terminus with "dimerization domains," which are composed of a hinge region linked to helixes. The helix was derived from either a helical segment from a parallel coiled coil of a leucine zipper or from a single helix in a designed four-helix bundle (98). The latter dimerization domain results in the generation of bivalent scFv fragments with higher avidity than the former, and this has been suggested to be due to better association of the four-helix bundle in dilute solutions or better spatial orientation of the scFv fragments for binding because of the different directionality of the four-helix bundle versus the leucine zipper (98). More recently, a different approach toward the generation of a dimerization domain was used by McGregor and colleagues (99), who tagged scFv fragments with Ig Cκ domains to drive their association into dimers.

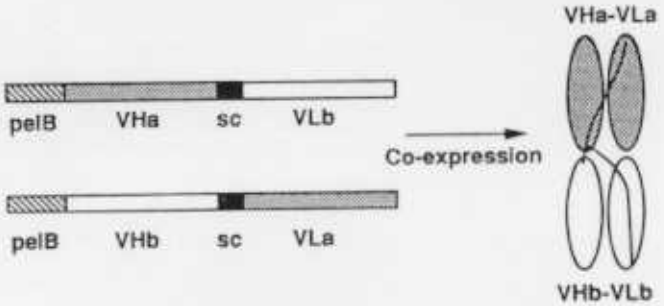

FIGURE 25.2 *Schematic representation of "diabodies" (90), bivalent scFv fragments that are produced by association of a heavy-chain variable (VH) domain from one scFv with a light-chain variable (VL) domain of another; a and b represent two different specificities, and expression of the hybrid scFv fragments as shown can produce a bispecific diabody made up of Fv fragments with specificities a and b.*

3. Diabodies: the observation that scFv fragments with 15-amino-acid linker sequences have a tendency to dimerize, presumably because of steric strain in the monomeric scFv, suggested that dimer formation could be further enhanced by variation of the linker length. Bispecific scFv fragments have been produced by linking the VH and VL domains of two different antibodies to form "crossover" chains (90) (Fig 25.2). The linker length was reduced to 5, 10 amino acids and no linker (i.e., for the latter, direct linkage of the VH to the VL domain). Molecular modeling indicated that formation of dimers in the latter construct was sterically possible (90). For these bivalent fragments with different linker lengths, binding to the appropriate antigens was observed, and kinetic measurements indicated that the "no-linker" version has significantly lower off rates, demonstrating that the forced packing of the two scFv fragments can result in an advantageous (with respect to affinity) alteration in the binding site (90).

REBUILDING THE FVS AND FAB FRAGMENTS FOR EXPRESSION AS COMPLETE IMMUNOGLOBULINS

For use in therapy, it is frequently desirable to use IgGs with effector functions such as complement fixation and ADCC. Furthermore, the Fc region of the antibody confers long serum persistence on the IgG molecule (100). The extremely short half-lives of Fv and Fab fragments in serum makes them unattractive for use if long half-lives are required, for example, in the treatment of tumors. To circumvent the problem of short half-life, higher doses and continuous infusion are necessary and from an economic standpoint this is unattractive.

The human IgG1 isotype has been identified as the isotype of choice for building antibodies with high activity in complement fixation and ADCC in therapy (35, 36). Glycosylation of the Fc is necessary for binding to FcRγ1 (101), and for this reason the host for expression of the complete antibodies should be capable of recognizing the N-linked glycosylation site on the C_H2 domain. A wide range of hosts, including insect cells (102) and tobacco plants (103), are now available for the expression of complete glycosylated antibodies (104).

Using toxins as effector functions

As an alternative to the Fc region, toxins can be linked to the recombinant Fv or Fab fragments and the resulting chimeras expressed in *E. coli*. However, these Fv or Fab-toxin chimeras have the disadvantage that they are cleared quickly, and, although it may be possible to overcome this by linking a dimeric C_H2 domain to the construct (105, 106), the recombinant protein then approaches the size of a complete antibody. In this case, it may, therefore, be more straightforward to attach the toxin chemically to the complete antibody after expression in one of the host cells previously mentioned, and such immunotoxins are now being used in clinical trials (107).

EXPRESSION OF FC FRAGMENTS IN *ESCHERICHIA COLI*

Escherichia coli can be used to secrete recombinant Fc fragments of the murine IgG1 isotype. These fragments bind to staphylococcal protein A (SpA) and have the same biologic half-life as the complete glycosylated IgG1 molecule (37), indicating that they are correctly folded. Pharmacokinetic analyses of Fc derivatives that have been mutated at the C_H2-C_H3 domain interface have resulted in the identification of the region of the murine IgG1 molecule that controls the catabolic rate (37). This region, designated the catabolic site, overlaps with the SpA-binding site (8) and, more recently, the site involved in binding to the neonatal transfer receptor, FcRn (108, 109). The residues that are involved in catabolism control are conserved in both human and murine isotypes (110) and are distinct from those involved in binding to FcRs (111–114) and C1q (115). This indicates that it will be possible to modulate the clearance rates of therapeutic antibodies by mutagenesis without affecting the other effector functions of the IgG molecule.

THE T-CELL RECEPTOR

The majority of TCRs are made up of a polymorphic $\alpha\beta$ heterodimer, and a much smaller proportion are $\gamma\delta$ TCRs, the function of which is less clear (116). Molecular modeling has been used to demonstrate that the extracellular regions of the invariably membrane-bound TCR resemble an Ig Fab (117–119). However,

the nature of the $\alpha\beta$ TCR–antigen interaction differs in a fundamental way to that of antibody and antigen. TCRs recognize peptide antigens bound to the groove of a class I (120) or class II (121) major histocompatibility molecule and are, therefore, MHC restricted (122) (Fig 25.3). These peptides are generally derived from intracellularly expressed antigen or exogenous antigen for class I and class II presentation, respectively. Thus, $\alpha\beta$ TCRs recognize sequential epitopes, whereas antibodies can recognize sequential and conformational or discontinuous epitopes.

Both experimental (123) and theoretical (sequence) (124) analyses indicate that $\alpha\beta$ TCRs recognize antigen presenting in a different way to $\gamma\delta$ TCRs (123) and the $\gamma\delta$ TCR–antigen interaction resembles that of an antibody with antigen (123). The recognition of transfectomas by two $\gamma\delta$ T-cell clones is not dependent on the class I or class II pathways (123), and the rules for $\gamma\delta$ TCR recognition are as yet unclear. Thus, despite the interesting features of $\gamma\delta$ TCRs, in the following sections the discussion is limited to the better characterized $\alpha\beta$ TCRs.

Expression of soluble T-cell receptors

The proposed structural similarities shared by the extracellular domains of TCRs and a Fab fragment (117–119) suggested that it would be straightforward to express TCRs in soluble form. However, this has proved not to be the case, and only after years of effort have systems for the efficient expression of TCRs in both mammalian and prokaryotic systems been described (125–135). The reasons for the difficulty in "solubilizing" the TCR are not clear (136), but the report that the binding specificity of a TCR cannot be transferred to an antibody by grafting of the putative CDR loops of TCRs onto an Ig variable domain framework (137) indicates that there are distinct structural differences between TCRs and Igs. That this is indeed the case has recently been demonstrated by the solution of the x-ray crystallographic structures of a Vβ-Cβ fragment (138, 138a) and a Vα domain (139, 139a) derived from two distinct TCRs.

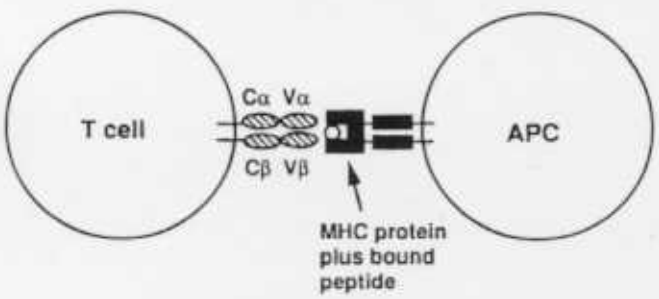

FIGURE 25.3 *T-cell recognition of antigenic peptide complexed with class I or class II major histocompatibility complex (MHC) proteins on the surface of an antigen-presenting cell (APC). The four extracellular domains of the TCR are represented by hatched ellipsoids.*

Mammalian systems

Initial attempts to express TCRs in soluble form by linking the Vα or Vβ domain to the Ig C_H1 or CL domain were unsuccessful in producing heterodimers (125). Vα-Cκ dimers that may resemble light-chain dimers can be expressed in high yields (126), and Vβ-Cβ fragments that bind to superantigens can be secreted in the absence of CD3 components (127). However, for studies of TCR recognition, the $\alpha\beta$ heterodimer is of foremost interest. The first report of the expression of a TCR heterodimer in soluble form using mammalian cells involved the production of Vβ-Cβ and Vα-Cα polypeptides as a phosphatidylinositol-linked dimer that could be cleaved from the cell surface of the CH0 cell transfectants using phospholipase C (128). Subsequently, soluble TCRs have been expressed as secreted Vα-Cα-Cκ:Vβ-Cβ-Cκ heterodimers from myeloma cells (129) and linked to the ζ chain of the CD3 complex in a basophilic leukemic line (130). In the Vα-Cα-Cκ:Vβ-Cβ-Cκ heterodimers, it is likely that the α-β association is driven by the Cκ domains. The yields of the TCRs vary from one system to another, but it is currently unclear whether this is due to differences in TCR sequences or the nature of the system being used.

Use of *Escherichia coli*

The reports of expression of secreted Fv and Fab fragments using *E. coli* as a host suggested that these systems could be extended to the production of soluble TCRs. However, to date only two systems have been described that result in the production of TCR V regions in soluble, secreted form (131, 132, 132a). To stabilize the Vα-Vβ domain interaction, the Vα and Vβ domains are linked by a 15-amino-acid linker peptide that is the same as that used by Huston and colleagues (85) for the production of an scFv fragment. In several reports, the single-chain TCRs (scTCRs, Vα linked to Vβ domain by a peptide, with the Vβ domain either at the C-terminus or N-terminus of the Vα domain) are isolated, denatured, and refolded (133–135). In the study of Novotny and colleagues (133), it was necessary to mutate several exposed hydrophobic residues to more hydrophilic ones to obtain a refolded TCR that is soluble. This scTCR has been shown to bind to fluorescein-MHC, which is the same specificity as that of the parent hybridoma (this TCR has antihapten specificity rather than the more conventional antipeptide specificity). Site-directed mutagenesis has been used to define the interaction of the soluble TCR with cognate antigen (140), and residues in the putative CDR loops appear to

be involved, indicating structural similarities between antibody and TCR recognition.

AFFINITY OF THE T-CELL RECEPTOR–PEPTIDE–MAJOR HISTOCOMPATIBILITY COMPLEX INTERACTION

To date, several mammalian-expressed TCRs have been used in studies to determine the affinity of the interaction of TCRs with cognate peptide-MHC (141–143) and, in one case, with superantigen (144). In all cases, the affinity of the TCR–peptide–MHC interaction is low (of the order of 5×10^{-5}–10^{-6} M), and this explains the difficulty in detecting the interaction of a soluble monovalent TCR with peptide-MHC. In vivo, the TCR-peptide-MHC interaction is stabilized by high valency and interactions of T-cell surface markers with cognate surface markers on antigen-presenting cells. It has been estimated that for a T cell to be activated, 60 to 300 TCR-peptide-MHC interactions are necessary per T cell (145, 146), indicating a highly multivalent interaction. The low affinity of TCRs is probably general (although there may be some exceptions) and suggests that, for use of TCRs as blocking or targeting agents in therapy, multivalency will be required.

WHAT CAN SOLUBLE T-CELL RECEPTORS BE USED FOR?

Generation of anti–T-cell receptor antibodies

Studies in T-cell biology such as analyses of TCR repertoires at the expression level would be greatly assisted by the availability of a panel of antibodies that are specific for TCRs of particular V-region families. Before the expression of soluble TCRs, the production of anti-TCR antibodies was hindered by the lack of purified protein for use as an immunogen. This problem can be overcome by using, for example, transfectomas expressing hybrid human-murine TCRs (147) or TCR-CD3 ζ chain chimeras (148) to immunize mice. Alternatively, soluble TCRs (149–151; Popov S, personal communication, 1995) have been used and shown to be efficacious as immunogens to generate anti-Vα, Vβ domain antibodies that recognize native T cells. Such approaches have obvious applications in the production of anti–V-region family and anticlonotypic antibodies.

Uses in vaccination for therapy of T-cell mediated autoimmunity

Soluble TCRs also have uses as vaccinating reagents for the treatment of diseases that are mediated by oligoclonal populations of pathogenic T cells. Examples of such diseases are multiple sclerosis and rheumatoid arthritis, although it is not clear as to the extent of oligoclonality insofar as different V gene usages have been reported (152–157). Animal models for both these diseases are available, and the disease models are characterized by oligoclonal T-cell responses against immunodominant epitopes (158–160). The use of peptides derived from the putative CDRs of oligoclonal TCRs has been shown to prevent experimental allergic encephalomyelitis in Lewis rats (161, 162), but an independent study indicates that this approach can exacerbate disease (163). Recently, soluble Vα domains (163a) and scTCRs (Kumar V, Sercarz E, personal communication) have been shown to be effective in blocking murine collagen–induced arthritis and experimental allergic encephalomyelitis, respectively. Thus, the general efficacy of vaccination using TCR V regions or peptides thereof is currently an area of active investigation.

Structural studies and affinity measurements

Soluble TCRs have obvious uses in both high-resolution structural studies and in analyses of TCR-peptide-MHC interactions. To date, despite intensive effort, the structure of an $\alpha\beta$ TCR has not been reported, but progress toward this aim for individual Vβ-Cβ and Vα fragments has been described by Mariuzza and colleagues (138, 138a, 139, 139a). The reasons for the difficulty in crystallization of TCRs are not obvious but may be related to inhomogeneities in soluble TCR preparations because of heterogeneous glycosylation. For this reason, the use of material expressed by recombinant *E. coli* cells (131–135) offers clear advantages.

The study of TCR-peptide-MHC interactions is of key interest in a number of areas. For example, it is not clear whether the absolute affinity (as monovalent TCR-peptide-MHC complexes) of the interaction determines the outcome in terms of activation, anergy induction, deletion, or antagonism (164). Elegant experiments demonstrate that avidity plays a role in positive or negative selection (165–167), but these studies do not directly address questions concerning the role of the absolute affinity of a monovalent TCR-peptide-MHC interaction in determining the response of the T cell. Such questions become particularly relevant in the design of peptide analogues (reviewed in 168) for therapy or vaccination because affinity might be a predictive measure of efficacy.

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