Methods in Molecular Biology™

VOLUME 207

Recombinant Antibodies for Cancer Therapy

Methods and Protocols

Edited by

Martin Welschof

Jürgen Krauss

HUMANA PRESS

METHODS IN MOLECULAR BIOLOGY™

Recombinant Antibodies for Cancer Therapy

Methods and Protocols

Edited by

Martin Welschof

Axaron Bioscience AG, Heidelberg, Germany

and

Jürgen Krauss

National Institutes of Health,
National Cancer Institute, Frederick, MD

Humana Press  **Totowa, New Jersey**

Preface

Since the advent of hybridoma technology more than two decades ago, numerous antibodies have entered the clinical setting as potent therapeutic agents. Their repeated application in humans, however, is limited by the development of human antimouse antibodies (HAMA) in the recipient, leading to allergic reactions against the foreign murine protein and rapid neutralization. To circumvent these limitations many new antibodies have recently been tailored through recombinant antibody technology. The initial clinical data show encouraging results, thus demonstrating the potential of these new therapeutic agents.

The purpose of *Recombinant Antibodies for Cancer Therapy* is to present a collection of detailed protocols in recombinant antibody technology. It is primarily addressed to scientists working on recombinant antibodies as well as clinicians involved with antibody-based therapies. As with other volumes of this series, we placed the main focus on providing detailed protocols describing procedures step-by-step. Moreover, each protocol supplies a troubleshooting guide containing detailed information on possible problems and hints for potential solutions.

Antibody technology is a subject of constant and rapid change. This volume, therefore, does not attempt to cover all possible current experimental approaches in the field. Rather, we present carefully selected protocols, written by competent authors who have successfully verified the particular method described. Given our own professional backgrounds and interest in oncology, we chose to concentrate chiefly on therapeutic agents for cancer patients.

Recombinant Antibodies for Cancer Therapy: Methods and Protocols consists of five sections. First, concise reviews give an overview of the current status of recombinant antibodies in cancer therapy, and the generation of antibody molecules through antibody engineering. This is followed by protocols grouped according to subject into four sections: Hybridoma-Derived Recombinant Antibodies, Recombinant Antibody Fragments from Phagemid-Displayed Antibody Repertoires, Antibody Fragments with Additional Properties, and Large Scale Production of Recombinant Antibodies for Clinical Applications.

We would like to commend all contributing authors for the high quality and clarity of their respective manuscripts. We thank them for sharing their extensive experience in dealing with intricate experimental problems. Moreover, we are

indebted to Prof. John Walker for his enthusiasm and encouragement, and Humana Press for publishing this volume. On a more personal level we are grateful to Mona, Lisa, Joshua, and Michaela for their patience and support.

Martin Welschof
Jürgen Krauss

Contributors

HINRICH ABKEN • *Tumorgenetik und Zellbiologie, Klinik I für Innere Medizin, University of Cologne, Germany*

LING-LING AN • *MedImmune, Inc., Gaithersburg, MD*

MICHAELA ARNDT • *National Cancer Institute at Frederick, Frederick, MD*

DANIÈLE ARNOLD-SCHILD • *Department of Immunology, Institute for Cell Biology, University of Tübingen, Germany*

ITAI BENHAR • *Department of Molecular Microbiology and Biotechnology, Tel-Aviv University, Ramat Aviv, Israel*

YEVGENY BERDICHEVSKY • *Department of Molecular Microbiology and Biotechnology, Tel-Aviv University, Ramat Aviv, Israel*

LIAT BINYAMIN • *Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel*

MICHAEL BRAUNAGEL • *Affitech AS, Oslo, Norway*

OLE H. BREKKE • *Affitech AS, Oslo, Norway*

SILVANA CANEVARI • *Unit of Molecular Therapies, Department of Experimental Oncology, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano, Italy*

PARTHA S. CHOWDHURY • *Human Genome Sciences Inc., Rockville, MD*

CHRISTIANE CHRIST • *Department of Transplantation Immunology, Institute for Immunology, University of Heidelberg, Germany*

RIKKE CLAUSEN • *Department of Pharmacology, The Royal Danish School of Pharmacy, Copenhagen, Denmark*

CYRIL J. COHEN • *Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel*

FRANCESCO COLOTTA • *Dompè Research Center, L'Aquila, Italy*

GALIT DENKBERG • *Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel*

JAN ENGBERG • *Department of Pharmacology, The Royal Danish School of Pharmacy, Copenhagen, Denmark*

MARIANGELA FIGINI • *Istituto Nazionale per lo Studio e la Cura dei Tumori, Department of Experimental Oncology, Unit of Molecular Therapies, Milano, Italy*

此为试读,需要完整PDF请访问: www.ertongbook.com

MARTINA FINGER • *Department of Transplantation Immunology, Institute for Immunology, University of Heidelberg, Germany*

RAINER FISCHER • *Fraunhofer Institute for Molecular Biology and Applied Ecology and Institut für Biologie VII, RWTH Aachen, Germany*

HANS HEINRICH FÖRSTER • *Labor Diagnostik GmbH, Leipzig, Germany*

JUNICHIRO FUTAMI • *Department of Bioscience and Biotechnology, Faculty of Engineering, University of Okayama, Japan*

ANDREW GREEN • *Unit of Molecular Therapies, Department of Experimental Oncology, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano, Italy*

HALLDIS HELLEBUST • *Affitech AS, Oslo, Norway*

INGRID HERMES • *Recombinant Antibody Unit (D0500), German Cancer Research Center, Heidelberg, Germany*

CLAUDIA HEUSER • *Tumorgenetik und Zellbiologie, Klinik I für Innere Medizin, University of Cologne, Germany*

ANDREAS HOMBACH • *Tumorgenetik und Zellbiologie, Klinik I für Innere Medizin, University of Cologne, Germany*

PETER J. HUDSON • *CRC for Diagnostic Technologies, CSIRO Health Sciences and Nutrition, Parkville, Australia*

LISELOTTE B. JENSEN • *Department of Pharmacology, The Royal Danish School of Pharmacy, Copenhagen, Denmark*

VALÉRIE JÉRÔME • *Vectron Therapeutics AG, Marburg, Germany*

TARRAN JONES • *AERES Biomedical Ltd., London, UK*

ARMIN KELLER • *Department of Technology, Axaron Bioscience AG, Heidelberg, Germany*

SERGEY M. KIPRIYANOV • *Affimed Therapeutics AG, Heidelberg, Germany*

CHRISTIAN KLEIST • *Department of Transplantation Immunology, Institute for Immunology, University of Heidelberg, Germany*

ROLAND E. KONTERMANN • *Vectron Therapeutics AG, Marburg, Germany*

PERNILLE KOPS • *Department of Pharmacology, The Royal Danish School of Pharmacy, Copenhagen, Denmark*

TINA KORN • *Institut für Molekularbiologie und Tumorforschung, University of Marburg, Marburg, Germany*

ALEXANDER A. KORTT • *CSIRO Health Sciences and Nutrition, Parkville, Australia*

JÜRGEN KRAUSS • *National Cancer Institute at Frederick, Frederick, MD*

DIANNE L. NEWTON • *SAIC-Frederick, National Cancer Institute, Frederick, Frederick, MD*

REVITAL NIV • *Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel*

SIOBHAN O'BRIEN • *AERES Biomedical Ltd., London, UK*

GERHARD OPELZ • *Department of Transplantation Immunology, Institute for Immunology, University of Heidelberg, Germany*

MICHAEL PFREUNDSCHUH • *Universitätskliniken des Saarlandes, Innere Medizin I, Homburg, Germany*

DANIEL PLAKSIN • *Molecular Biology Group, Peptor Ltd., Kiryat Weizmann, Israel*

BARBARA E. POWER • *CSIRO Health Sciences and Nutrition, Parkville, Australia*

HANS-GEORG RAMMENSEE • *Department of Immunology, Institute for Cell Biology, University of Tübingen, Germany*

YORAM REITER • *Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel*

ERIK RIISE • *Department of Pharmacology, The Royal Danish School of Pharmacy, Copenhagen, Denmark*

DALE RUBY • *SAIC-Frederick, National Cancer Institute, Frederick, MD*

SUSANNA M. RYBAK • *National Cancer Institute at Frederick, Frederick, MD*

HANSJÖRG SCHILD • *Department of Immunology, Institute for Cell Biology, University of Tübingen, Germany*

DINA SEGAL • *Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel*

PETER SØRENSEN • *Department of Pharmacology, The Royal Danish School of Pharmacy, Copenhagen, Denmark*

PETER TERNESS • *Department of Transplantation Immunology, Institute for Immunology, University of Heidelberg, Germany*

JOHN E. THOMMESEN • *Division of Cell Biology, Department of Biology, University of Oslo, Norway*

BARBARA UCHANSKA-ZIEGLER • *Institut für Immungenetik, Universitätsklinikum Charité, Humboldt-Universität zu Berlin, Germany*

CARMEN VAQUERO-MARTIN • *Institut für Biologie VII, RWTH Aachen, Germany*

GEORGE VASMATZIS • *Cancer Center and Division of Experimental Pathology, Mayo Clinic, Rochester, MN*

MARTIN WELSCHOF • *Department of Technology, Axaron Bioscience AG, Heidelberg, Germany*

ANNA M. WU • *Department of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, CA*

HERREN WU • *MedImmune Inc., Gaithersburg, MD*

PAUL J. YAZAKI • *Department of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, CA*

ALI F. YENIDUNYA • *Department of Pharmacology, The Royal Danish School of Pharmacy, Copenhagen, Denmark*

ANDREAS ZIEGLER • *Institut für Immungenetik, Universitätsklinikum Charité, Humboldt-Universität zu Berlin, Germany*

Contents

此为试读,需要完整PDF请访问: www.ertongbook.com

I

Introduction

1

Generation of Antibody Molecules Through Antibody Engineering

Sergey M. Kipriyanov

1. Introduction

Twenty-five years ago, Georges Köhler and César Milstein invented a means of cloning individual antibodies, thus opening up the way for tremendous advances in the fields of cell biology and clinical diagnostics *(1)*. However, in spite of their early promise, monoclonal antibodies (MAbs) were largely unsuccessful as therapeutic reagents resulting from insufficient activation of human effector functions and immune reactions against proteins of murine origin. These problems have recently been overcome to a large extent using genetic-engineering techniques to produce chimeric mouse/human and completely human antibodies. Such an approach is particularly suitable because of the domain structure of the antibody molecule *(2)*, where functional domains carrying antigen-binding activities (Fabs or Fvs) or effector functions (Fc) can be exchanged between antibodies (*see* **Fig. 1**).

On the basis of sequence variation, the residues in the variable domains (V-region) are assigned either to the hypervariable complementarity-determining regions (CDR) or to framework regions (FR). It is possible to replace much of the rodent-derived sequence of an antibody with sequences derived from human immunoglobulins without loss of function. This new generation of "chimeric" and "humanized" antibodies represents an alternative to human hybridoma-derived antibodies and should be less immunogenic than their rodent counterparts. Furthermore, genetically truncated versions of the antibody may be produced ranging in size from the smallest antigen-binding unit or Fv through Fab' to $F(ab')_2$ fragments. More recently it has become possible to produce totally human recombinant antibodies derived either from antibody libraries *(3)* or single immune B cells *(4)*, or from transgenic mice bearing human immunoglobulin loci *(5,6)*.

From: *Methods in Molecular Biology, vol. 207: Recombinant Antibodies for Cancer Therapy: Methods and Protocols*
Edited by: M. Welschof and J. Krauss © Humana Press Inc., Totowa, NJ

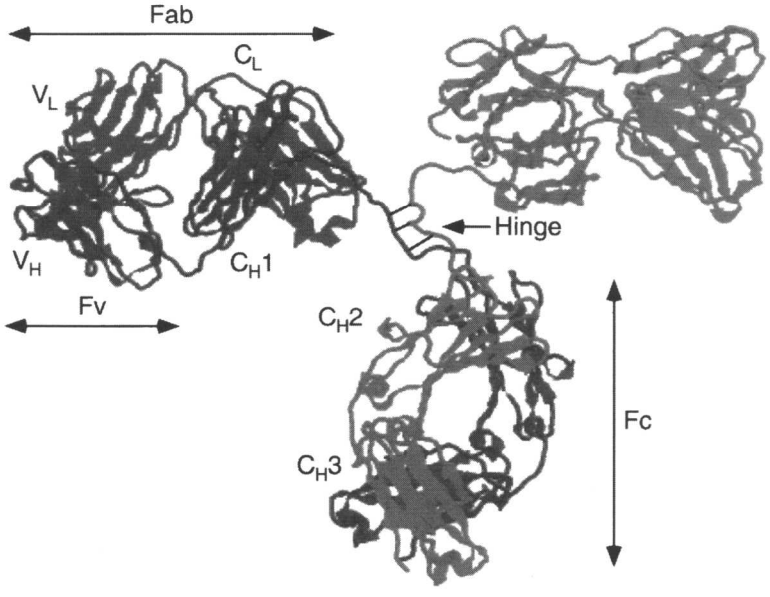

Fig. 1. Domain organization of an IgG molecule. Antigen-binding surface is formed by variable domains of the heavy (V_H) and light (V_L) chains. Effector functions are determined by constant C_H2 and C_H3 domains. The picture is based on the crystal structure of an intact IgG2 anti-canine lymphoma MAb231 *(2)* (pdb entry 1IGT). The drawing was generated using a molecular visualization program RasMac Molecular Graphics, version 2.7.1 (R. Sayle, Biomolecular Structure, Glaxo Research and Development, Greenford, Middlesex, UK).

2. Cloning the Antibody Variable Regions

Significant progress has been made in the in vitro immunization of human B cells *(7)* and in the development of transgenic mice containing human immunoglobulin loci (for review, *see* **refs. *5,8***). Recombinant DNA technology can also be employed for generating human MAbs from human lymphocyte mRNA. The genetic information for antibody variable regions is generally retrieved from total cDNA preparations using the polymerase chain reaction (PCR) with antibody-specific primers ***(9,10)***. As a source of immunoglobulin-specific mRNA, one can use hybridoma cells ***(11)***, human peripheral blood lymphocytes (PBL) ***(3)***, and even a single human B cell ***(4,12)***. Using the latter approach, it is possible to avoid the cumbersome hybridoma technology and obtain human antibody fragments with the original V_H/V_L pairing. Single bacterial colonies expressing antigen-specific antibody fragments can be identified by colony screening using antigen-coated membranes ***(13)***. Novel high-throughput selection technologies allow screening thousands of different antibody clones at a time ***(14)***. The appropriate V_H/V_L combination may also be selectively enriched from a phage-displayed antibody library through a series of immunoaffinity steps referred to as "library panning" ***(15,16)***.

3. Genetically Engineered Monoclonal Antibodies

3.1. Chimeric Antibodies with Human Constant Regions

The first generation of recombinant monoclonal antibodies consisted of the rodent-derived V-regions fused to human constant regions (**Fig. 2**). It is thought that the most immunogenic regions of antibodies are the conserved constant domains *(17)*. Because the antigen-binding site of the antibody is localized within the variable regions, the chimeric molecules retain their binding affinity for the antigen and acquire the function of the substituted constant regions. The human constant regions allow more efficient interaction with human complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) effector mechanisms. Rituximab (Rituxan; IDEC Pharmaceuticals, San Diego, and Genentech, Inc., San Francisco, CA) is a chimeric anti-CD20 MAb containing the variable regions of the CD20-binding murine IgG1 MAb, IDEC-2B8, as well as human IgG1 and kappa constant regions *(18,19)*. Rituximab was the first monoclonal antibody to be approved for therapeutic use for any malignancy. Its approval was based on a single-agent pivotal trial in patients with indolent B-cell lymphoma, in which 166 patients were enrolled from 31 centers in the United States and Canada. Administration of this antibody induced remissions in 60% of patients with relapsed follicular lymphomas, including 5%–10% complete remissions *(20)*.

As a further step to reduce the murine content in an antibody, procedures have been developed for humanizing the Fv regions.

3.2. Antibody Humanization (Reshaping)

3.2.1. Humanization by CDR Grafting

CDRs build loops close to the antibody's N-terminus, where they form a continuous surface mounted on a rigid scaffold provided by the framework regions. Crystallographic analyses of several antibody/antigen complexes have demonstrated that antigen-binding mainly involves this surface (although some framework residues have also been found to take part in the interaction with antigen). Thus, the antigen-binding specificity of an antibody is mainly defined by the topography and by the chemical characteristics of its CDR surface. These features in turn are determined by the conformation of the individual CDRs, by the relative disposition of the CDRs, and by the nature and disposition of the side chains of the amino acids comprising the CDRs *(21)*.

A large decrease in the immunogenicity of an antibody can be achieved by grafting only the CDRs of xenogenic antibodies onto human framework and constant regions *(22,23)* (**Fig. 2**). However, CDR grafting *per se* may not result in the complete retention of antigen-binding properties. Indeed, it is frequently found that some framework residues from the original antibody need to be preserved in the humanized molecule if significant antigen-binding affinity is to be recovered *(24,25)*. In this case, human V regions showing the greatest sequence homology to murine V regions are chosen from a database in order to provide the human framework. The selection of human FRs can be made either from human consensus sequences or from individual human antibodies. In some rare examples, simply transferring CDRs onto the most identical human

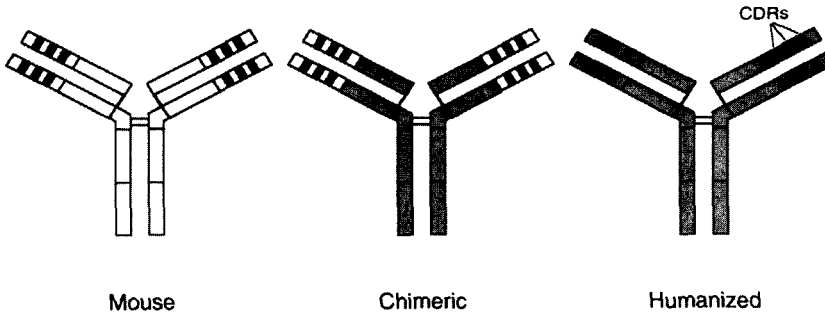

Fig. 2. Humanization of an IgG molecule. The mouse sequences are shown in white and the human sequences are shown in gray. In a chimeric antibody, the mouse heavy- and light-chain variable region sequences are joined onto human heavy-chain and light-chain constant regions. In a humanized antibody, the mouse CDRs are grafted onto human V-region FRs and expressed with human C-regions.

V-region frameworks is sufficient for retaining the binding affinity of the original murine MAb *(26)*. However, in most cases, the successful design of high-affinity CDR-grafted antibodies requires that key murine residues be substituted into the human acceptor framework to preserve the CDR conformations. Computer modeling of the antibody is used to identify such structurally important residues that are then included in order to achieve a higher binding affinity. The process of identifying the rodent framework residues to be retained is generally unique for each reshaped antibody and can therefore be difficult to foresee.

Such approach was successfully used for humanizing a MAb 4D5 against the product of protooncogene HER2 *(27)*. HER2 is a ligand-less member of the human epidermal growth factor receptor (EGFR) or ErbB family of tyrosine kinases. HER2 overexpression is observed in a number of human adenocarcinomas and results in constitutive HER2 activation. Specific targeting of these tumors can be accomplished with antibodies directed against the extracellular domain of the HER2 protein. The MAb 4D5, has been fully humanized and is termed trastuzumab (Herceptin; Genentech, San Francisco, CA). Treatment of HER2-overexpressing breast cancer cell lines with trastuzumab results in a number of phenotypic changes, such as downmodulation of the HER2 receptor, inhibition of tumor cell growth, reversed cytokine resistance, restored E-cadherin expression levels, and reduced vascular endothelial growth factor production. Interaction of trastuzumab with the human immune system via its human IgG1 Fc domain may potentiate its anti-tumor activities. In vitro studies demonstrate that trastuzumab is very effective in mediating antibody-dependent cell-mediated cytotoxicity against HER2-overexpressing tumor targets *(28)*. Trastuzumab treatment of mouse xenograft models results in marked suppression of tumor growth. When given in combination with standard cytotoxic chemotherapeutic agents, trastuzumab treatment generally results in statistically superior anti-tumor efficacy compared with either agent given alone *(28)*.

此为试读,需要完整PDF请访问: www.ertongbook.com