Oliver Kayser, Rainer H. Müller

Pharmaceutical Biotechnology

Drug Discovery and Clinical Applications

WILEY-VCH Verlag GmbH & Co. KGaA

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Edited by
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Edited by

Dr. Oliver Kayser
Free University Berlin
Institute of Pharmacy
Pharmaceutical Technology
Biopharmacy & Biotechnology
Kelchstr. 31
12169 Berlin
Germany

Prof. Dr. Rainer H. Müller
Free University Berlin
Institute of Pharmacy
Pharmaceutical Technology
Biopharmacy & Biotechnology
Kelchstr. 31
12169 Berlin
Germany

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data. A catalogue record for this book is available from the British Library.

Bibliographic information published by Die Deutsche Bibliothek Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at http://dnb.ddb.de.

Printed in the Federal Republic of Germany
Printed on acid-free paper.

Composition: Laserwords Private Ltd, Chennai, India
Printing: betz-druck GmbH, Darmstadt
Bookbinding: Litges & Dopf Buchbinderei GmbH, Heppenheim
ISBN 3-527-30554-8

Preface

Pharmaceutical biotechnology has a long tradition and is rooted in the last century, first exemplified by penicillin and streptomycin as low molecular weight biosynthetic compounds. Today, pharmaceutical biotechnology still has its fundamentals in fermentation and bioprocessing, but the paradigmatic change affected by biotechnology and pharmaceutical sciences has led to an updated definition. Upon a suggestion by the European Association of Pharma Biotechnology (EAPB), pharmaceutical biotechnology is defined as a science covering all technologies required for the production, manufacturing, and registration of biotechnological drugs.

The biopharmaceutical industry has changed dramatically since the first recombinant protein (Humulin®) was approved for marketing in 1982. The range of resources required for the pharmaceutical industry has expanded from its traditional fields. Advances in the field of recombinant genetics allows scientists to routinely clone genes and create genetically modified organisms that can be used in industrial production processes. Also, specific therapeutic proteins can be synthesized in nonbiological ways, and recombinant proteins can be isolated from complex mixtures in commercially viable processes. In contrast to academic research, industrial development and manufacturing is guided by cost and time effectiveness, patent protection, exclusivity periods, and regulatory compliance. There are many critical industry issues that companies have to face; hence there is a need for new pharmaceutical biotechnology textbooks focussing on industrial needs.

Therapeutic proteins and the recently approved antisense oligonucleotide Fomivirsen® represent new and innovative biotech drugs that are different from classical drugs in the development and production process. In this area, pharmaceutical companies are confronted with new challenges to develop new products and to apply new technologies. Industrial needs are particularly different and are either not discussed or are only marginally discussed in existing textbooks, which is why we feel that there is a need for a new pharmaceutical biotechnology textbook.

We asked experts from the pharmaceutical biotech area to present their integrated view to answer questions focussing on industrial needs in the discovery and manufacture of recombinant drugs and new therapies. We are glad that a majority of contributors, active in the pharmaceutical industry, have participated and shared their views on new developments in protein production, production organisms, DNA vaccines, bioinformatics, and legal aspects. Distinct problems related to recombinant proteins that

have arisen in recent years, such as drug stability, pharmacokinetics, and metabolization, are discussed in detail. It should be mentioned that for the first time the topic of generic recombinant drugs is presented in this textbook.

Biotechnology is a fast-moving area and crucial topics for future technologies can be recognized today. We wanted to give an insight into these future enterprise technologies and had asked for contributions to highlight new developments in gene therapy, tissue engineering, personalized medicine, and xenotransplantation having a realistic chance of being used in industrial applications.

In this textbook, you will find updated facts and figures about the biotech industry, product approvals, and discussions of how biotechnology is applied in human and animal health care, and in industrial and environmental processes. We address how biotech is being employed in national security efforts as well as the ethical issues that are frequently debated when people discuss the use of biotechnology in health sciences.

We would like to thank all contributors for their contributions, because we know that time was short and most of the papers were written alongside their regular duties. Special thanks to Dr. Andrea Pillmann, Wiley VCH, for her support in the layout, proofreading, and production of this textbook.

We are convinced that this textbook is filling a niche and covering industrial needs and interests in the pharmaceutical biotech area. Our point of view is that this textbook will cater to scientists and decision makers in pharmaceutical and biotechnological companies, venture capitals/finance, and politics.

O. Kayser **Berlin, December 2003**
R.H. Müllers

Foreword

Pharmaceutical Biotechnology is a multidisciplinary scientific field undergoing an explosive development. Advances in the understanding of molecular principles and the existence of many regulatory proteins have established biotechnological or therapeutic proteins as promising drugs in medicine and pharmacy. More recent developments in biomedical research highlight the potential of nucleic acids in gene therapy and antisense RNAi technology that may become a medical reality in the future.

The book attempts to provide a balanced view of the biotechnological industry, and the number of experts from the industry sharing their knowledge and experience with the readers gives the book an outstanding value. All contributors provide with each chapter an up-to-date review on key topics in pharmaceutical biotechnology. Section 1 serves as an introduction to basics in protein production and manufacturing. Particular emphasis not only on production organisms like microorganisms and plants but also on industrial bioprocessing will be appreciated by the reader.

The advent and development of recombinant proteins and vaccines is described in detail in Part 2. Biotech drugs have created a number of unique problems because of their mostly protein nature. The production, downstream processing, and characterization is in many aspects different from conventional low molecular weight drugs and is highlighted by selected experts still in touch with the lab bench. Bringing the therapeutic protein to the patient is a major challenge. Protein formulation, biopharmaceutical aspects, and drug regulation are fields that are fast developing and well recognized by their new and innovative techniques. Drug regulation has a major impact on the whole drug manufacturing process, which is why special chapters on the drug approval process in Europe and the United States, and biogenerics are of high interest. Finally, in Part 4, experts provide an outlook on potential drugs and therapeutic strategies like xenotransplantation that are under investigation. Hopefully, some of these concepts will find clinical application in the following years.

I believe that there is a distinct need for a pharmaceutical biotech book focusing on the industrial needs of recombinant drugs and providing detailed insight into industrial processes and clinical use. Therefore, this work is not only a valuable tool for the industrial expert but also for all pharmacists and scientists from related areas who wish to work with biotech drugs. In life-learning courses and the professional environment, this compact book is the basis for a solid understanding for those who wish to gain a better overview of the industry they are working in.

Robert Langer
MIT Boston, November 2003

Contents

List of Contributors

Dr. Albrecht F. Kiderlen
Robert Koch-Institut
Nordufer 20
13353 Berlin
Germany

Prof. Dr. Andreas Bechthold
Albert-Ludwigs-Universität Freiburg
Pharmazeutische Biologie
Stefan-Meier-Straße 19
79104 Freiburg
Germany

Dr. Antonio J. Grillo-López
Neoplastic and Autoimmune Diseases
Research Institute
P. O. Box 3797
Rancho Santa Fe, CA 92067
USA

Prof. Dr. Bernd Meibohm
Department of Pharmaceutical Sciences
College of Pharmacy, University of
Tennessee, Health Science Center
Memphis, TN 38163
USA

Prof. Dr. David B. Resnik
The Brody School of Medicine
East Carolina University
Greenville, NC 27858
USA

Prof. Dr. Dirk Hoffmeister
The University of Wisconsin
School of Pharmacy
777 Highland Avenue
Madison, WI 53705
USA

Dr. Erno Pungor
Berlex Biosciences
2600 Hilltop Drive
Richmond, CA 94804
USA

Dr. Gary Walsh
Industrial Biochemistry Program
University of Limerick
Limerick City
Ireland

Prof. Dr. Gregory J. Brunn
Transplantation Biology and the Departments of Pharmacology and Experimental Therapeutics
Mayo Clinic
Rochester, MI 55905
USA

Prof. Dr. Hartmut Derendorf
Department of Pharmaceutics, College of Pharmacy
University of Florida
Gainesville, FL 32610
USA

Prof. Dr. Irmgard Merfort
Albert-Ludwigs-Universität Freiburg
Pharmazeutische Biologie
Stefan-Meier-Straße 19
79104 Freiburg
Germany

Dr. Janet Patton
Bone Research Unit
Medical Research Council/
University of the Witwatersrand
7 York Road
Parktown 2193 Johannesburg
South Africa

Prof. Dr. Jeffrey L. Platt
Transplantation Biology and the Departments of Pharmacology and Experimental Surgery, Immunology and Pediatrics
Mayo Clinic
Rochester, MI 55905
USA

Dr. Jeffrey Ulmer
Chiron Corporation
4560 Horton Street
Emeryville, CA 94608-2916
USA

Dr. Jens-Peter Gregersen
Chiron-Behring GmbH
Postfach 1630
35006 Marburg
Germany

Dr. John Donnelly
Chiron Corporation
4560 Horton Street
Emeryville, CA 94608-2916
USA

Dr. Jörg Knäblein
Schering AG
Analytical Development Biologicals
Müllerstraße 178
13342 Berlin
Germany

June Teare
Bone Research Unit
Medical Research Council/
University of the Witwatersrand
7 York Road
Parktown 2193 Johannesburg
South Africa

Dr. Jutta Haunschild
MorphoSys AG
Lena-Christ-Strasse 48
82152 Martinsried
Germany

Prof. Dr. Klaus Cichutek
Paul-Ehrlich-Institut
Paul-Ehrlich-Straße 51–59
63225 Langen
Germany

Dr. Lentsha Nathaniel Ramoshebi
Bone Research Unit
Medical Research Council/
University of the Witwatersrand
7 York Road
Parktown 2193 Johannesburg
South Africa

Louise Renton
Bone Research Unit
Medical Research Council/
University of the Witwatersrand
7 York Road
Parktown 2193 Johannesburg
South Africa

Priv. Doz. Dr. Oliver Kayser
Freie Universität Berlin
Institut für Pharmazie
Pharmazeutische Technologie
Biopharmazie & Biotechnologie
Kelchstraße 31
12169 Berlin
Germany

Prof. Dr. Rainer H. Müller
Freie Universität Berlin
Institut für Pharmazie
Pharmazeutische Technologie
Biopharmazie & Biotechnologie
Kelchstraße 31
12169 Berlin
Germany

Priv. Doz. Dr. Ralf Lipp
Schering AG
Müllerstraße 178
13342 Berlin
Germany

Dr. Stefan Pelzer
Combinature Biopharm AG
Robert-Rössle-Straße 10
13125 Berlin
Germany

Thato Matsaba
Bone Research Unit
Medical Research Council/
University of the Witwatersrand
7 York Road
Parktown 2193 Johannesburg
South Africa

Dr. Titus Kretzschmar
MorphoSys AG
Lena-Christ-Strasse 48
82152 Martinsried
Germany

Dr. Udo Gottschalk
Bayer AG
GB Pharma-Biotechnologie
Friedrich-Ebert-Straße 217
42096 Wuppertal
Germany

Dr. Ugo Ripamonti
Bone Research Unit
Medical Research Council/
University of the Witwatersrand
7 York Road
Parktown 2193 Johannesburg
South Africa

Dr. Walter Hinderer
BioGeneriX AG
Janderstraße 3
68199 Mannheim
Germany

Color Plates

Fig. 2.1 Photography of a sporulated *Streptomyces* strain growing on solid medium. The blue drops indicate the production of an antibiotic (aromatic polyketide).




Major technology	Mammalian (CHO) cells	Transgenic mammal milk	Transgenic chicken eggs	Transgenic plants
Companies	Amgen (Thousand Oaks, CA) Genentech (S. San Francisco, CA) other current biologics manufacturers; Crucell (Leiden, Netherlands) uses human cells	GTC Biotherapeutics (Framingham, MA) PPL Therapeutics (Edinburgh, UK) BioProtein (Paris, France)	Avigenics (Athens, GA) Origen Therapeutics (Burlingame, CA) TranXenoGen (Shrewsbury, MA) Viragen (Plantation, FL) GeneWorks (Ann Arbor, MI) Vivalis (Nantes, France)	Croptech (Blacksburg, VA) Epicyte (San Diego, CA) Large Scale Biology (Owensboro, KY) Meristem Therapeutics (Clermont-Ferrand, France) Prodigene (College Station, TX)
Estimated cost (cost/g raw material)*	$150	$1–$2	$1–$2	$0.05

*Company estimates

Fig. 3.2 Companies and technologies in biomanufacturing. A comparison of different expression systems shows the big differences in terms of costs, ranging from US$150 per gram for CHO cells to US$0.05 per gram for transgenic plants [11].

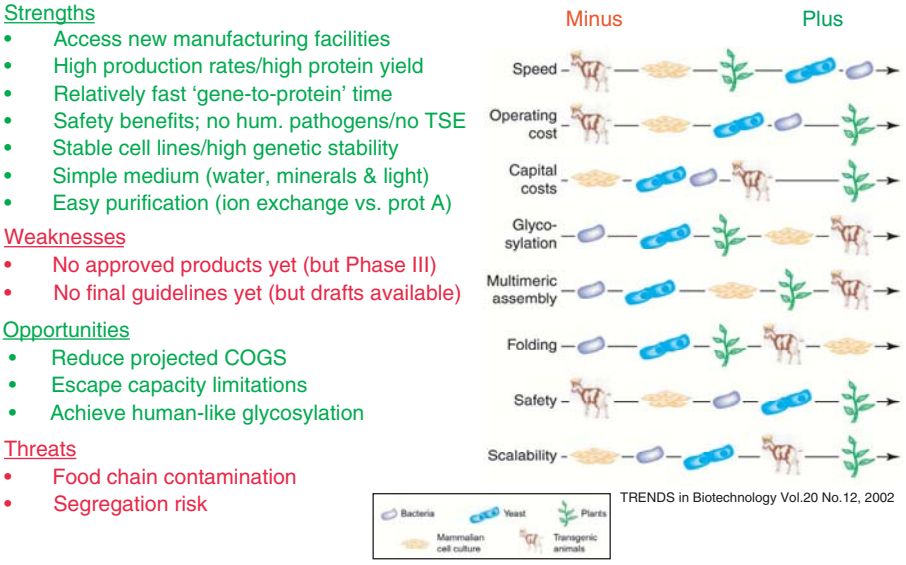


Fig. 3.3 SWOT analysis of plant expression systems. Plant expression systems have a lot of advantages (plus) over other systems and are therefore mostly shown on the right-hand side of the picture (Raskin I et al., Plants and human health in the twenty-first century. *Trends in Biotechnol.* **2002** *20*, 522–531.). Herein different systems (transgenic animals, mammalian cell culture, plants, yeast, and bacteria) are compared in terms of speed (how quickly they can be developed), operating and capital costs and so on, and plants are obviously advantageous. Even for glycosylation, assembly and folding, where plants are not shown on the right-hand side (meaning other systems are advantageous), some plant expression systems are moving in that direction (as will be shown exemplarily in the section for moss). Also, the weaknesses and threats can be dealt with, using the appropriate plant expression system [20].

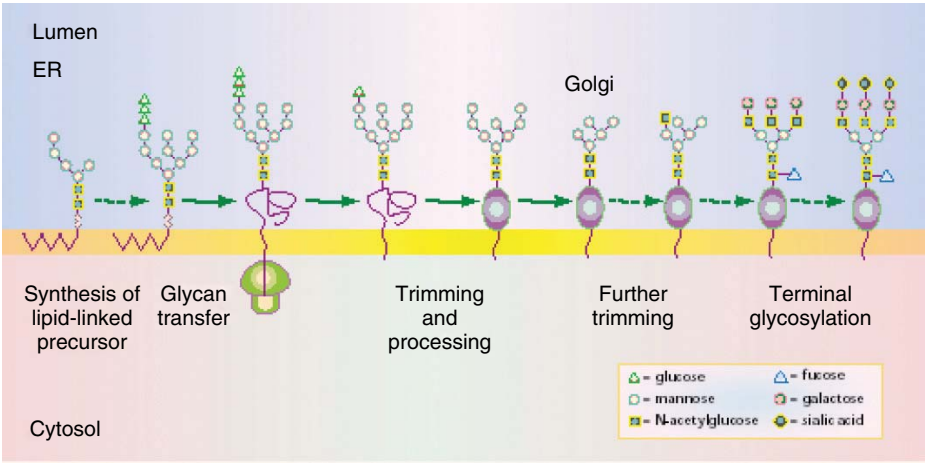


Fig. 3.4 The glycosylation pathway via ER and Golgi apparatus. In the cytosol carbohydrates are attached to a lipid precursor, which is then transported into the lumen of the ER to finish core glycosylation. This glycan is now attached to the nascent, folding polypeptide chain (which is synthesized by ribosomes attached to the cytosolic side of the ER from where it translocates into the lumen) and subsequently trimmed and processed before it is folded and moved to the Golgi apparatus. Capping of the oligosaccharide branches with sialic acid and fucose is the final step on the way to a mature glycoprotein [23].

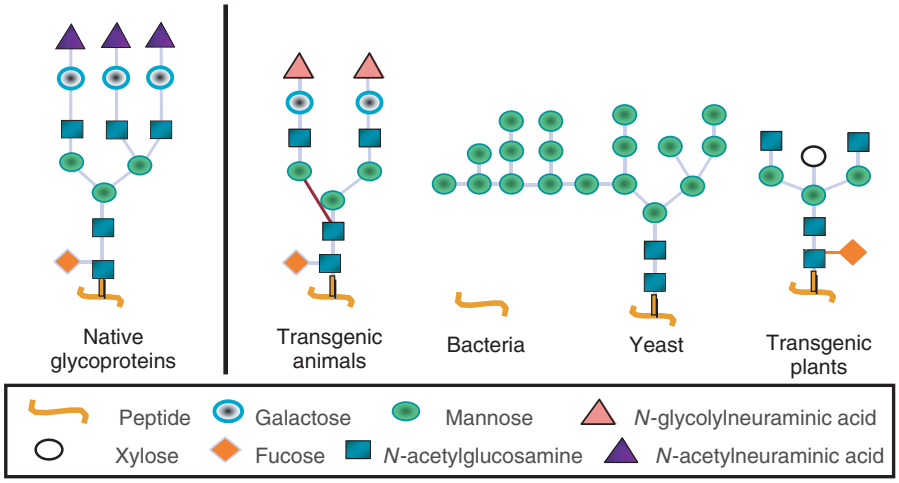


Fig. 3.5 Engineering plants to humanlike glycosylation. The first step to achieve humanlike glycosylation in plants is to eliminate the plant glycosylation pattern, that is, the attachment of β-1-2-linked xylosyl and α-3-linked fucosyl sugars to the protein. Because these two residues have allergenic potential, the corresponding enzymes xylosyl and fucosyl transferase are knocked out. In case galactose is relevant for the final product, galactosyl transferase is inserted into the host genome. Galactose is available in the organism so that this single-gene insertion is sufficient to ensure galactosylation [24].

Fig. 3.6 Secretion of the biopharmaceuticals via tobacco roots. The tobacco plants are genetically modified in such a way, that the protein is secreted via the roots into the medium (''rhizosecretion''). In this example, the tobacco plant takes up nutrients and water from the medium and releases GFP (green fluorescent protein). Examination of root-cultivation medium by its exposure to near-ultraviolet illumination reveals the bright green-blue fluorescence characteristics of GFP in the hydroponic medium (left flask in panel lower left edge). The picture also shows a schematic drawing of the hydroponic tank, as well as tobacco plants at different growth stages, for example, callus,–fully grown and greenhouse plantation [24].

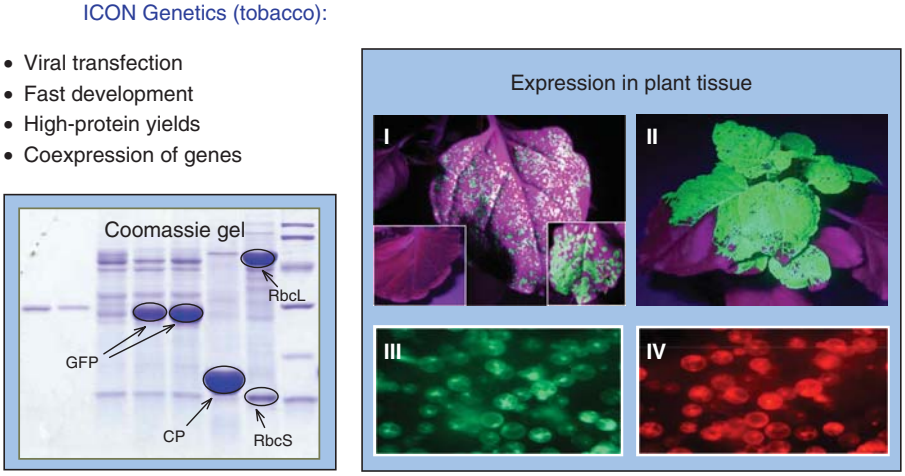


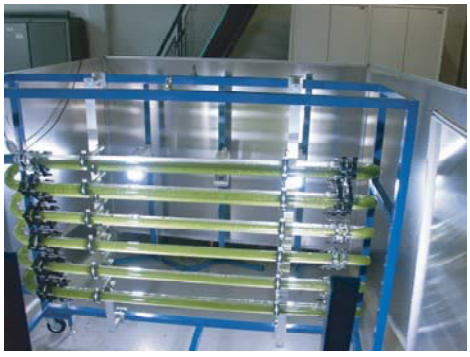
Fig. 3.7 Viral transfection of tobacco plants. This new generation platform for fast (1 to 2 weeks), high-yield (up to 5 g per kilogram of fresh leaf weight) production of biopharmaceuticals is based on proviral gene amplification in a non-food host. Antibodies, antigens, interferons, hormones, and enzymes could successfully be expressed with this system. The picture shows development of initial symptoms on a tobacco following the agrobacterium-mediated infection with viral vector components that contain a *GFP* gene (I); this development eventually leads to a systemic spread of the virus, literally converting the plant into a sack full of protein of interest within two weeks (II). The system allows to coexpress two proteins in the same cell, a feature that allows expression of complex proteins such as full-length monoclonal antibodies. Panel III and IV show the same microscope section with the same cells, expressing green fluorescent protein (III) and red fluorescent protein (IV) at the same time. The yield and total protein concentration achievable are illustrated by a Coomassie gel with proteins in the system: GFP (protein of interest), CP (coat protein from wild-type virus), RbcS and RbcL (small and large subunit of ribulose-1,5-bisphosphate carboxylase) [24].

Greenovation (moss system):

- Simple medium (photoautotrophic plant needs only water and minerals)
- Robust expression system (good expression levels from 15 to 25 °C)
- Secretion into medium via human leader sequence (broad pH range: 4–8)
- Easy purification from low-salt medium via ion exchange
- Easy genetic modifications to cell lines
- Stable cell lines/high genetic stability
- Codon usage like human (no changes required)
- Inexpensive bioreactors from the shelf
- Nonfood plant (no segregation risk)
- Good progress on genetic modification of glycosylation pathways (plant to human)

Fig. 3.8 Greenovation use a fully contained moss bioreactor. This company has established an innovative production system for human proteins. The system produces pharmacologically active proteins in a bioreactor, utilizing a moss (*Physcomitrella patens*) cell culture system with unique properties [24].

30 L pilot reactor for moss



Two weeks after incubation

Fig. 3.11 Scaling of photobioreactors up to several 1000 L. The moss bioreactor is based on the cultivation of *Physcomitrella patens* in a fermenter. The moss protonema is grown under photoautotrophic conditions in a medium that consists essentially of water and minerals. Light and carbon dioxide serve as the only energy and carbon sources. Cultivation in suspension allows scaling of the photobioreactors up to several 1000 L. Adaptation of existing technology for large-scale cultivation of algae is done in cooperation with the Technical University of Karlsruhe. Courtesy of greenovation Biotech GmbH (Freiburg, Germany) and Professor C. Posten, Technical University (Karlsruhe, Germany).

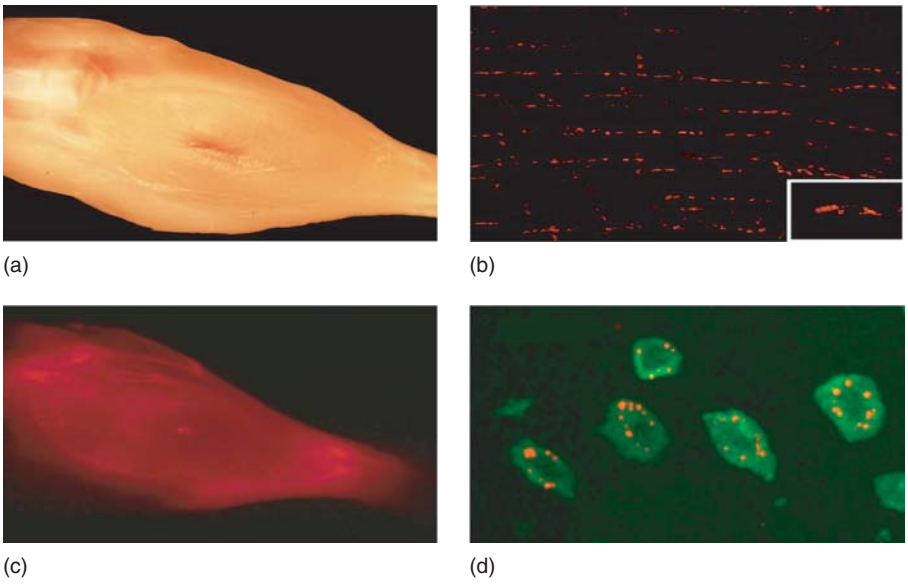


Fig. 5.3 Distribution of injected DNA vaccines. A rhodamine-conjugated DNA vaccine was injected into a tibialis anterior muscle of a mouse shown by light (panel A) and fluorescence (panel C) microscopy (~5× magnification). A longitudinal section of the muscle is shown in panel B (~250× magnification), demonstrating the presence of DNA in cells between the muscle fibers. Panel C shows the phagosomal location of the plasmid DNA (in red) within the cells isolated from the injected tissues (~2500× magnification).

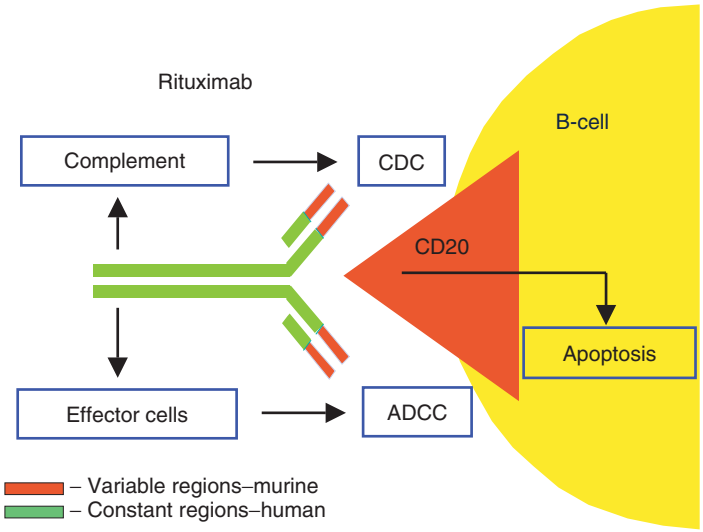


Fig. 12.1 Mechanism of action of rituximab. The chimeric (mouse/human) antibody, rituximab, binds to the CD20 antigen on B-cells and (a) activates complement to effect CDC, (b) attracts effector cells via Fc receptors to effect ADCC, and (c) transmits a signal into the cell to induce apoptosis.

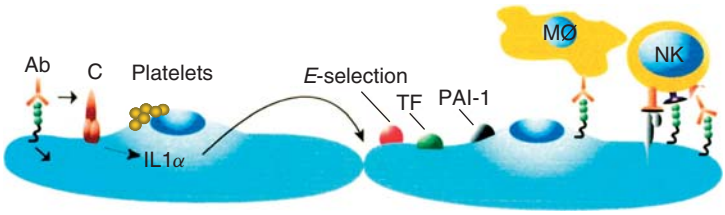


Fig. 15.3 Pathogenesis of acute vascular rejection. Activation of endothelium by xenoreactive antibodies (Ab), complement (C), platelets, and perhaps by inflammatory cells (natural killer (NK) cells and macrophages (M⊘) leads to the expression of new pathophysiologic properties. These new properties, such as the synthesis of tissue factor (TF) and plasminogen activator inhibitor type 1 (PAI-1), promote coagulation; the synthesis of *E*-selectin and cytokines such as IL1α promote inflammation. These changes in turn cause thrombosis, ischemia, and endothelial injury, the hallmarks of acute vascular rejection. (Adapted from *Nature* 1998: **392**(Suppl.) 11–17, with permission.)

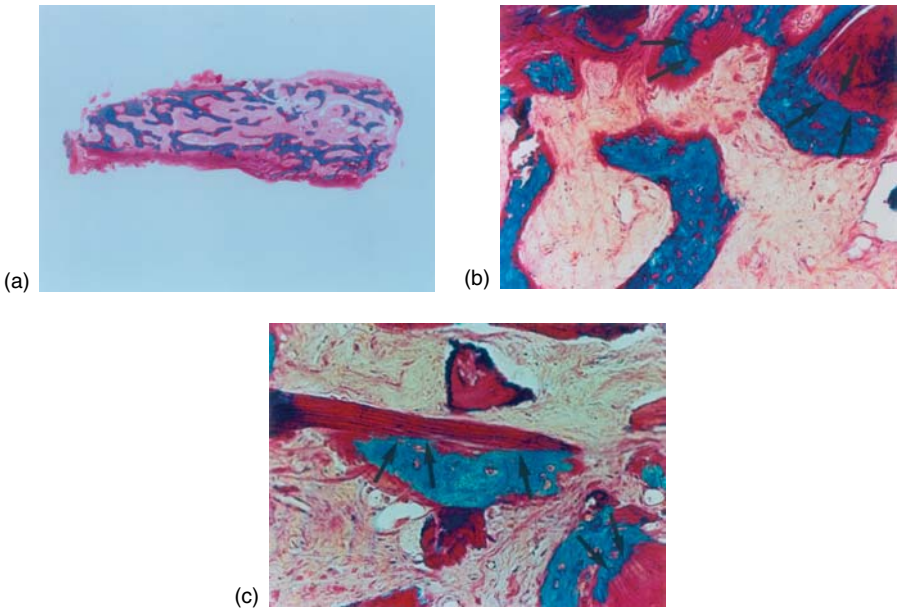


Fig. 16.2 Photomicrographs of tissue induction and morphogenesis in bioptic material 90 days after implantation of naturally derived BMPs/OPs purified from bovine bone matrix in human mandibular defects. (a) Trabeculae of newly formed mineralized bone covered by continuous osteoid seams within highly vascular stroma. (b) and (c) High-power views showing cellular mineralized bone surfaced by osteoid seams. Newly formed and mineralized bone directly opposing the implanted collagenous matrix carrier (arrows) confirms bone formation by induction. Undecalcified sections at 7 µm stained with Goldner's trichrome. Original magnification: (a) ×14; (b) ×40; and (c) ×50.

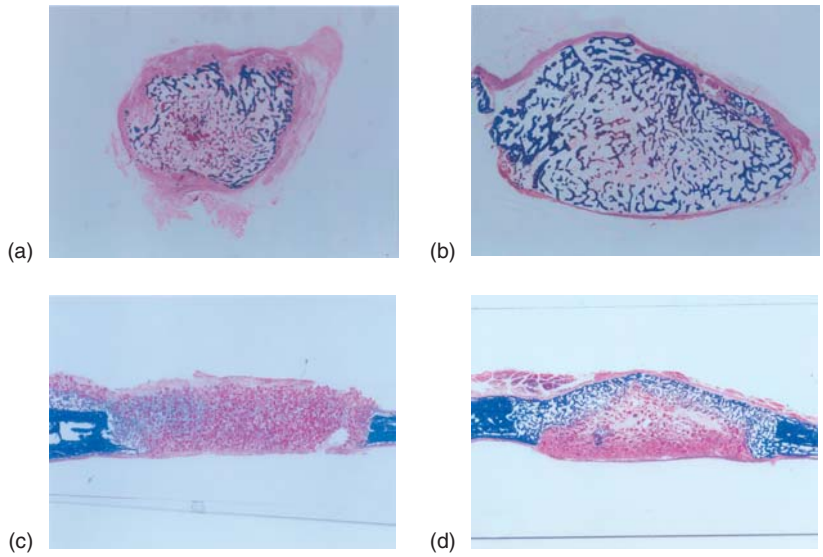


Fig. 16.4 Tissue morphogenesis and site–tissue-specific osteoinductivity of recombinant human-transforming growth factor-β2 (hTGF-β2) in the adult primate *Papio ursinus*. (a and b) Endochondral bone induction and tissue morphogenesis by hTGF-β2 implanted in the *rectus abdominis* muscle and harvested (a) 30 and (b) 90 days after heterotopic implantation. Heterotopic bone induction by a single administration of (a) 5- and (b) 25-µg hTGF-β2 delivered by 100 mg of guanidinium-inactivated collagenous matrix. (c and d) Calvarial specimens harvested from the same animals as shown in (a and b). (c) Lack of bone formation in a calvarial defect 30 days after implantation of 10-µg hTGF-β2 delivered by collagenous bone matrix. (d) Osteogenesis, albeit limited, is found in a specimen treated with 100-µg hTGF-β2 with bone formation only pericranially 90 days after implantation. Note the delicate trabeculae of newly formed bone facing scattered remnants of collagenous matrix particles, embedded in a loose and highly vascular connective tissue matrix. Original magnification: (a and b) $\times$4.5; (c and d) $\times$3. Undecalcified sections cut at 4 µm stained with Goldner's trichrome.

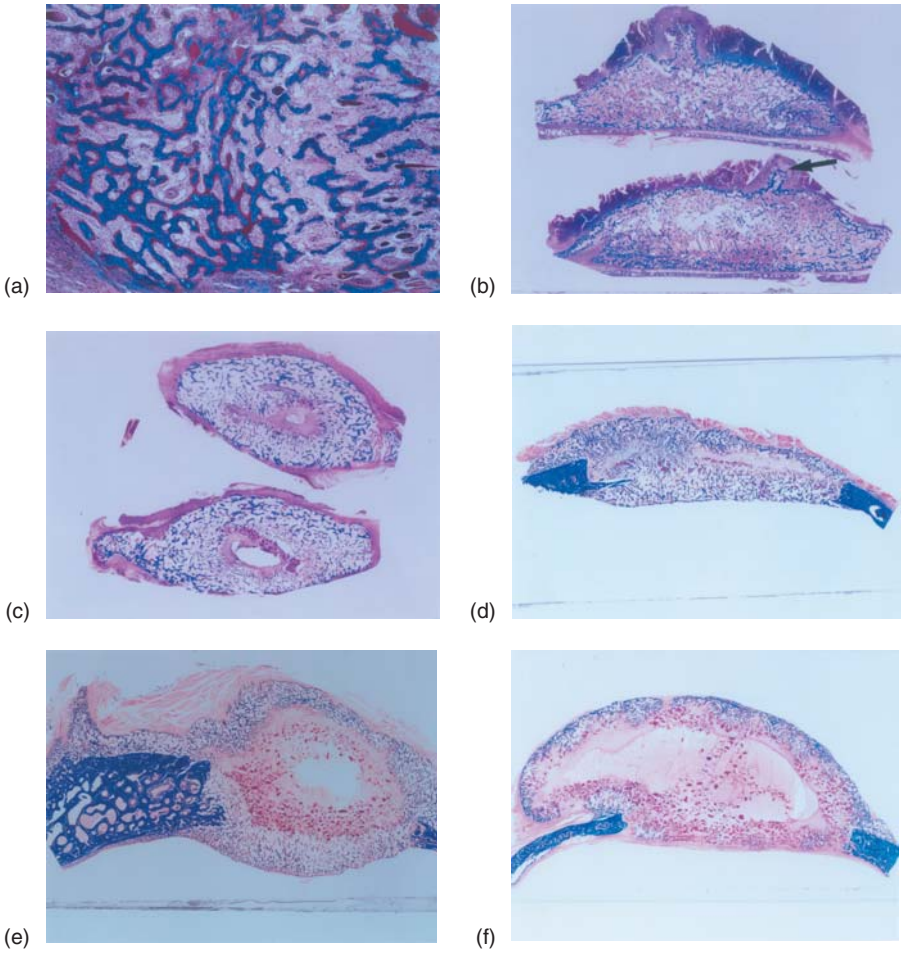


Fig. 16.6 Synergistic tissue morphogenesis and heterotopic bone induction by the combinatorial action of recombinant human osteogenic protein-1 (hOP-1) and transforming growth factor-β1 (hTGF-β1). (a) Rapid and extensive induction of mineralized bone in a specimen generated by 25-µg hOP-1 combined with 0.5-µg hTGF-β1 on day 15. Mineralized trabeculae of newly formed bone are covered by osteoid seams populated by contiguous osteoblasts. (b and c) Photomicrographs of massive ossicles that had formed between the muscle fibers and the posterior fascia of the *rectus abdominis* using binary applications of 25- and 125-µg hOP-1 interposed with 5-µg hTGF-β1 on day 30. Corticalization of the large heterotopic ossicles with displacement of the *rectus abdominis* muscle and extensive bone marrow formation permeating trabeculae of newly formed bone. Arrow in (b) points to a large area of chondrogenesis protruding within the rectus abdominis muscle. (d, e, and f) Low-power photomicrographs of calvarial defects treated by binary applications of 100-µg hOP-1 and 5 µg of naturally derived TGF-β1 purified from porcine platelets as described [55] and harvested on day 30. The calvarial specimens show extensive bone differentiation with pronounced vascular tissue invasion and displacement of the calvarial profile 30 days after implantation of the binary morphogen combinations. Original magnification: (a) $\times$30; (b, c) $\times$3.5; (d, e, and f) $\times$3. Undecalcified sections cut at 4 µm and stained with Goldner's trichrome.

Part I
Introduction to Concepts and Technologies in Pharmaceutical Biotechnology