PROGRESS IN

Nucleic Acid Research and Molecular Biology

edited by

KIVIE MOLDAVE

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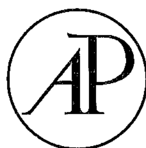
Volume 60



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IMMUNOGEN 2167, pg. 1
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Academic Press
a division of Harcourt Brace & Company
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
http://www.apnet.com

Academic Press Limited
24-28 Oval Road, London NW1 7DX, UK
http://www.hbuk.co.uk/ap/

International Standard Book Number: 0-12-540060-8

PRINTED IN THE UNITED STATES OF AMERICA
98 99 00 01 02 03 BB 9 8 7 6 5 4 3 2 1

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Contents

SOME ARTICLES PLANNED FO

Bacillus thuringiensis Ins
Molecular Mode of Acti

Francis Rajamohan, Mi

I. *Bacillus thuringiensis:* Th
II. Insecticidal Activity and T
III. Structural Architecture of
IV. Molecular Mode of Action
V. Pros and Cons of Current
VI. Mechanism of Insect Resi
References . . . .

Transcription Activation
of a Regulator with the
The Model of Phage φ2

Fernando Rojo, Mario
and Margarita Salas

I. Introduction . . . .
II. Transcription Regulation
III. Promoter Context Can In
between a Regulator and
References . . . .

Eukaryotic Translation E
Structure, Expression, F
in Aminoacyl-tRNA Cha

B. S. Negrutskii and A.

I. Introduction . . . .
II. Structural Features of EF

IMMUNOGEN 2167, pg. 2
Phigenix v. Immunogen
IPR2014-00676

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Development of More Efficacious Antibodies for Medical Therapy and Diagnosis

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Bethesda, Maryland 20892

I. Introduction 169
II. Procedure for Reducing the Immunogenicity of a Nonhuman Antibody to a Minimum 172
  A. Sample Design of a "Humanization" Protocol by SDR Transfer 182
III. Generation of Multivalent/Multispecific Antibodies 183
  A. Sample Construction of a Bispecific Molecule Using Two Single-Chain Fvs Dimerized via Electrostatically Complementary Fc Regions 188
  B. Sample Construction of a Tetraspecific Molecule from Two Bispecific Single-Chain Diabodies Dimerized via Electrostatically Complementary Fc Regions 190
IV. Conclusion 190
  References 192

Two procedures for improving the efficacy of medically important antibodies are described. The first procedure is designed to reduce the immunogenicity of nonhuman antibodies to the barest minimum—the "humanization" is accomplished by transplanting only the specificity-determining residues of the nonhuman antibody onto a human antibody template. The second procedure is designed to permit the easy production of multispecific/multivalent antibodies via heterodimer formation of electrostatically complementary Fc regions. © 1998 Academic Press

I. Introduction

Antibodies represent a major factor in our defense against invading pathogens and noxious substances. Antibodies are generated to bind specifically to the foreign substance (antigen) and to neutralize it and facilitate its

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Phigenix v. Immunogen
IPR2014-00676

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elimination by normal biological processes. Antibodies are multivalent (at least bivalent) molecules, thus they can cross-link antigens, thereby immobilizing them. The binding of antibodies to antigens also may cause the recruitment of other molecules or of certain cells—additional components of the immune system—which would then act to dispose of the invading substance or organism.

All antibodies share the same basic structural unit that consists of two identical heavy chains (MW of each, ~50,000 to ~77,000) and two identical light chains (MW of each, ~25,000). Each light chain is usually linked to a heavy chain by a disulfide bond and the heavy chains are usually linked together by one or more disulfide bonds. Each chain has variable and constant domains. The N-terminal domain of both light and heavy chains is variable and is followed by one constant domain in the light chain (C_L) and by three or four constant domains in the heavy chain (C_H1, C_H2, C_H3, and C_H4) depending on antibody class.

The antibody class, or isotype, is determined by the constant domains. The light chain exists in two distinct isotypes called kappa (κ) and lambda (λ). The heavy chain may be α, γ, δ, ϵ, or μ type, which defines the antibody class as IgA, IgG, IgD, IgE, or IgM, respectively. IgG is the major antibody class in human serum; IgE is the antibody responsible for allergic reactions. The constant domain of the heavy chain determines the effector function(s) of an antibody, e.g., complement activation, and Fc receptor binding. Different classes have different biological properties.

The variable region contains the antigen-binding site. Each variable domain consists of three hypervariable segments, called the complementarity-determining regions (CDRs) (*1*), flanked by four relatively less variable framework regions. The antigen-binding site is built mainly with CDR residues, with occasional contribution from neighboring framework residues. The lengths and sequences of the CDRs vary from antibody to antibody, resulting in different antigen-binding specifities. The V_L:V_H module is often referred to as the Fv fragment and the V_LC_L:V_HC_H1 as the Fab fragment. The C_H2 and C_H3 domains of the two heavy chains, plus the C_H4 in the case of IgE and IgM, constitute the Fc fragment.

The exquisite specificity of the binding of an antibody to its antigen and the ability of the immune system to respond to challenge by all sorts of antigens have found many uses in medical therapy and diagnosis. The use of antivenom against snake bites, antitoxins against bacterial infections, immune serum globulin against certain diseases, and so forth, are some of the well-known uses of specific antisera (see Ref. *2* for a review). Among the more recent uses of antibodies in medicine is the specific targetting of cells or tissues, e.g., tumor cells, either for location (*in vivo* imaging) or for destruction (*3*).

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IMMUNOGEN 2167, pg. 4
Phigenix v. Immunogen
IPR2014-00676

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With the advent of hybridoma technology (*4*), monoclonal antibodies of virtually any desired specificity can be produced. Further, the development of novel expression systems has permitted the generation of pure antibodies in large amounts. For various reasons, including ethical considerations, monoclonal antibodies are usually obtained from nonhuman sources. Unfortunately, the human immune system will react to, and attempt to eliminate, any nonhuman (or nonself) entity. This necessitates the "humanization," i.e., the reduction of the immunogenicity, of the nonhuman antibodies prior to their use in human patients, especially if the treatment protocol requires protracted use of such molecules. Various procedures have been devised to humanize nonhuman antibodies (*5–11*). Here, we present a new procedure, currently being developed in our laboratory, that seeks to reduce immunogenicity to the barest minimum while at the same time preserving the antigen-binding properties of the original antibody.

Another topic of interest is the generation of multispecific/multivalent antibodies. A molecule that can bind different ligands has many potential uses. For example, a molecule of a desired reactivity can be brought to close proximity to a target cell by using a bispecific antibody that can bind the molecule via one site and an antigen on the surface of the cell via the other. Likewise, two different cells can be brought together with the use of a bispecific antibody. Further, there are instances when an antigen has only one site (epitope) to which a given antibody type can bind. Such an antigen cannot be cross-linked by antibodies, the binding sites of which have the same binding properties; at least one other antibody type, with a different and nonoverlapping specificity, will be required to cross-link the antigen. In nature, several different antibody types are elicited by a single antigen (in a normal polyclonal response). If an antibody could be engineered so that its binding sites have different specificities, such an antibody could by itself produce the same effect as two, or more, different antibodies. Various techniques are currently in use for the generation of bispecific antibodies (*12–26*).

Multivalency amplifies the affinity of antibodies for their specific antigens. Usually, antibody–antigen reactions are characterized by nanomolar (or better) affinity constants, when the antigen is a protein. With carbohydrate antigens, the binding constants are often much lower. The binding of an antibody to its antigen can be improved by increasing the number of combining sites on the antibody (the antigen-binding sites) so that, even with a low intrinsic binding affinity per site, the avidity due to the presence of multiple sites can be substantial. This is observed in nature in antibodies of the IgM class, which can have as many as 12 identical antigen-binding sites.

Judicious engineering can generate multispecific and multivalent antibodies. Here, we present a procedure that we are developing for the easy generation of multispecific/multivalent molecules.

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