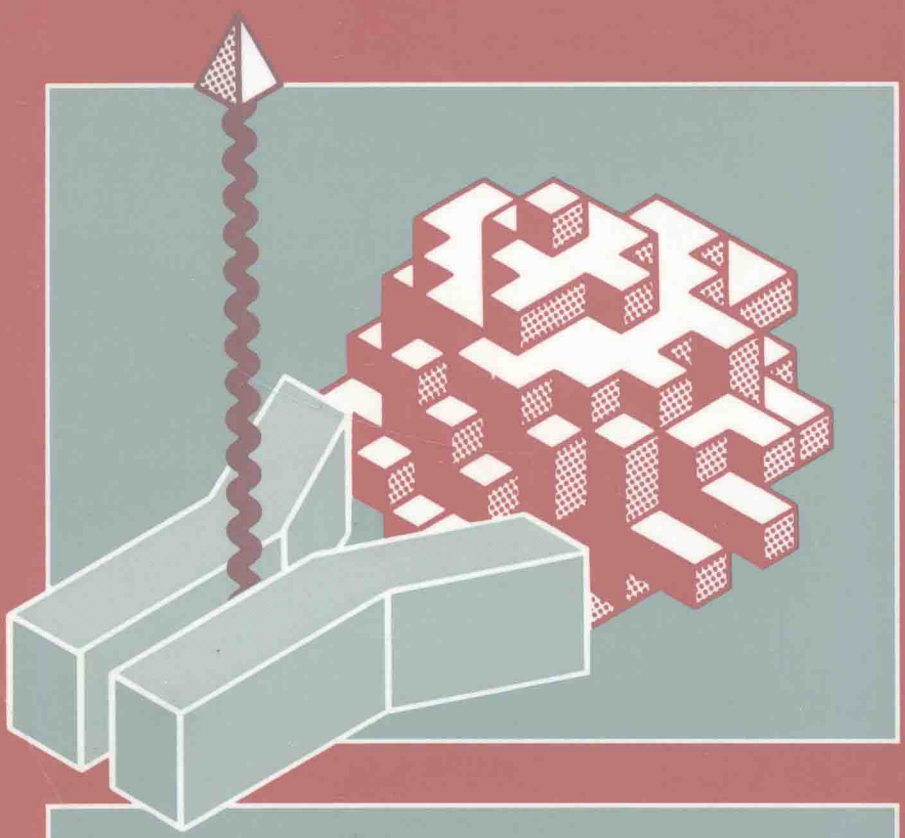
IMMUNOSCINTIGRAPHY

Practical Aspects and Clinical Applications



A. C. PERKINS and M. V. PIMM

Wiley-Liss

IMMUNOSCINTIGRAPHY

Practical Aspects and Clinical Applications

A. C. PERKINS

Department of Medical Physics
University Hospital
Queen's Medical Centre
Nottingham,
United Kingdom

M. V. PIMM

Cancer Research Campaign Laboratories
University of Nottingham
Nottingham,
United Kingdom

WILEY-LISS

A JOHN WILEY & SONS, INC., PUBLICATION
New York • Chichester • Brisbane • Toronto • Singapore

Address all Inquiries to the Publisher
Wiley-Liss, Inc., 41 East 11th Street, New York, NY 10003

Printed in United States of America

While the authors, editors, and publisher believe that drug selection and dosage and the specifications and usage of equipment and devices, as set forth in this book, are in accord with current recommendations and practice at the time of publication, they accept no legal responsibility for any errors or omissions, and make no warranty, express or implied, with respect to material contained herein. In view of ongoing research, equipment modifications, changes in governmental regulations and the constant flow of information relating to drug therapy, drug reactions and the use of equipment and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each drug, piece of equipment or device for, among other things, any changes in the instructions or indications of dosage or usage and for added warnings and precautions.

Recognizing the importance of preserving what has been written, it is a policy of John Wiley & Sons, Inc. to have books of enduring value published in the United States printed on acid-free paper, and we exert our best efforts to that end.

Library of Congress Cataloging-in-Publication Data

Perkins, A.C.
Immunoscintigraphy: practical aspects and clinical applications/
by A.C. Perkins and M.V. Pimm.
p. cm.
Includes bibliographical references and index.
ISBN 0-471-56072-3
1. Radioimmunoimaging. I. Pimm, M.V. II. Title.
[DNLM: 1. Antibodies, Monoclonal—diagnostic use. 2. Antibodies, Monoclonal—isolation & purification. 3. Isotope Labeling—methods. 4. Neoplasms—radionuclide imaging. QZ 241 P448i]
RC78.7.R38P47 1991
616.07'57—dc20
DNLM/DLC
for Library of Congress 90-13155
CIP

IMMUNOSCINTIGRAPHY

Practical Aspects and Clinical Applications

Preface

The use of radiolabelled antibodies in clinical diagnosis has had a slow evolution. Starting with a concept that originated at the beginning of the century, it has taken the fusion of previously unrelated scientific specialities to result in a technique that is only now capable of providing diagnostic information relevant to a broad range of clinical specialities. Much of the technology involved in the production of radiolabelled antibodies suitable for clinical administration is poorly understood by the majority of clinical and paramedical staff within hospital departments. This book is primarily intended to be a handbook of immunoscintigraphy aimed at nuclear medicine personnel such as nuclear medicine physicians, radiologists, medical physicists, radiopharmacists, technicians, and radiographers, who are now beginning to use antibodies for routine clinical investigations. It should also be of interest to workers in research laboratories and the radiopharmaceutical industry. We have not set out to review the entire scope of immunoscintigraphy but have emphasised the practical aspects of this rapidly developing field so as to provide a basis for the future developments that are now inevitable.

Acknowledgments

This book has arisen from ten years' practical experience of imaging with radiolabelled monoclonal antibodies. The authors would like to thank their friends and families for support with this work. In particular we would like to thank our colleagues in the departments of Cancer Research, Medical Physics, Radiology, Surgery, Obstetrics and Gynaecology, and Radiotherapy at University Hospital, Nottingham.

Commonly Used Abbreviations and Terms

Ab:	Antibody
AFP:	Alpha fetoprotein
APF:	Activated platelet factor
Ag:	Antigen
Bq:	Bequerel
CEA:	Carcinoembryonic antigen
Ci:	Curie
CT:	Computed tomography
Da:	Dalton
DTPA:	Diethylene triamine pentaacetic acid
DTPAA:	Diethylene triamine pentaacetic acid anhydride
EDTA:	Ethylenediaminetetraacetic acid
ELISA:	Enzyme-linked immunosorbent assay
EMA:	Epithelial membrane antigen
Fab:	Antigen-binding fragment (of antibody)
$F(ab')_2$:	Bivalent antigen-binding fragment
FACS:	Fluorescent activated cell sorter
Fc:	Crystallisable fragment (of antibody)
Fg:	Fragment
FDA:	Food and Drug Administration
GP:	Glycoprotein
HAHA:	Human anti-human antibody
HAMA:	Human anti-mouse antibody
HCG:	Human chorionic gonadotrophin
HIG:	Human immunoglobulin
HMFG:	Human milk fat globule
HMW:	High molecular weight
HMWA:	High-molecular-weight antigen
HPLC:	High-performance liquid chromatography
HSA:	Human serum albumin
IC:	Immune complex
Ig:	Immunoglobulin
IgG:	Gamma immunoglobulin
IgM:	Macro immunoglobulin
IS:	Immunoscintigraphy
LSqCC:	Lung squamous cell carcinoma
MAA:	Melanoma-associated antigen
MAB:	Monoclonal antibody
MBq:	Mega bequerel
MOAB:	Monoclonal antibody
NCA:	Normal cross-reacting antigen
NF:	Normalisation factor
NSLC:	Non small-cell lung carcinoma
OD:	Optical density
PBS:	Phosphate-buffered saline
PLAP:	Placental alkaline phosphatase
RAID:	Radioimmunodetection
RCP:	Radiochemical purity
RES:	Reticuloendothelial system
RIA:	Radioimmunoassay
RIS:	Radioimmunoscintigraphy
RIT:	Radioimmunotherapy

ROI:	Region of interest
SCLC:	Small-cell lung carcinoma
SDS-PAGE:	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SPECT:	Single photon emission computed tomography
SqCC:	Squamous cell carcinoma
TAA:	Tumour-associated antigen
TAG:	Tumour-associated glycoprotein
TLC:	Thin layer chromatography
TRIS:	Tris (hydroxymethyl) aminomethane

Contents

1 Production, Purification, and Quality Control of Antibodies for Immunoscintigraphy

ANTIBODIES

Antibodies, or immunoglobulins, are produced by the body in response to the presence of foreign materials (antigens). Antibodies possess specific binding regions which recognise the corresponding site or *determinant* on the antigen. Antibodies combine physically, rather than chemically, with the specific antigen against which they were induced. In certain cases this combination of antibody and antigen initiates a series of biological processes which result in destruction and elimination of the antigen.

An antigen can have several different determinants (epitopes) each of which can stimulate the production of a different antibody. This stimulation involves the activation of a class of lymphocytes called B lymphocytes. Each B lymphocyte has the ability to differentiate into a plasma cell which then secretes antibody. Each B lymphocyte produces a single type of plasma cell which produces a specific antibody against a single antigenic determinant. Thus if animals are immunised with an antigen, they produce and secrete into their blood a mixture of antibodies each against a different determinant on the antigen. These antibodies are derived from many different stimulated B lymphocytes and are a heterogeneous mixture of many different types of antibody. Because they are the product of many different individual populations, or clones, of B lymphocytes, they are referred to as polyclonal antibodies. Monoclonal antibodies are produced using cell culture techniques. These are homogeneous in nature being secreted by a clone from a single cell line.

Antibody Structure

Immunoglobulins (Igs) are proteins. There are five main classes of immunoglobulin, IgG, IgM, IgA, IgE, and IgD. Each class of antibody serves a different function of immunity and defence. The commonest immunoglobulins are IgG and IgM, and these are the types most frequently produced as a result of the monoclonal antibody procedure (see below).

The IgG antibodies are made up of two long and two short amino acid chains referred to as *heavy* (H) and *light* (L) chains.

Heavy chains have molecular weights of about 50,000 daltons and light chains of about 25,000, so the whole IgG molecule has a molecular weight of about 150,000 daltons. In mice there are four types of heavy chain. Both heavy (gamma) chains in each molecule are the same, and so there are four different types, or isotypes, of IgG, referred to as IgG1, IgG2a, IgG2b, and IgG3. The first three are the commonest type of immunoglobulin naturally produced by mice and therefore the commonest IgG isotypes produced as murine monoclonal antibodies. There are two types of light chain, referred to as *kappa* and *lambda*. Again each immunoglobulin's light chains are of the same type. The kappa is the predominant type and consequently the most frequently encountered in monoclonal antibodies.

The four chains of the immunoglobulin molecule are held together by disulphide bonds. Both heavy and light chains are folded into three or four loop and sheet-like structures termed domains. Thus the three-dimensional structure of the molecule is quite complex, but it is usual to depict the structure schematically as a Y shape shown in Figure 1.1. The combining site of the antibody, that is the part which recognises the antigen, is at the end of the molecule which has both light and heavy chains. This is the variable region of the immunoglobulin molecule, where variations in amino acid sequences occur in both the heavy and light chains and confer upon each antibody its unique antigen-binding properties. The combining site is formed by a combination of the terminal parts of both the heavy and light chains. Since there are two such structures in each IgG molecule, each has two combining sites, a property referred to as bivalency.

The basic structure of the IgM immunoglobulins is similar to that of IgG, with either kappa or lambda light chains and one sort of heavy chain (mu chains). Here, however, the molecule is formed essentially of five IgG-like subunits held together by another protein chain (the J chain), so that its overall molecular weight is about 900,000, and it has ten antigen-combining sites potentially available to react with antigen, although in practice, because of the size and shape of the molecule, each site may not be able to come into contact with the antigen.

Antibodies bind physically to the antigens which induced their formation. This interaction is essentially an ionic, non-covalent interaction, and is in fact an equilibrium between bound and free antibody and antigen. The Law of Mass Action applies to this interaction and the equilibrium constant, K from the following classic equation:

$$K = \frac{[AbAg]}{[Ab][Ag]}$$

is a measure of the strength of antibody–antigen interaction. This affinity, or more correctly the avidity of a monoclonal antibody, is expressed in units of litres/mol. Most antibodies have K values in the range 10^6–10^9 l/mol, but some have values up to 10^{12} l/mol. Generally it can be expected that the higher the K values, the better the binding of antibody will be to its target antigen.

Monoclonal Antibody Production

Throughout this century attempts have been made to identify specific antigens in human pathological lesions, especially tumours, which could be the targets for antibody therapy. The early attempts were carried out by immunisation of animals with human tissues (mainly tumour cells). These procedures may have produced antibodies with some degree of specificity for

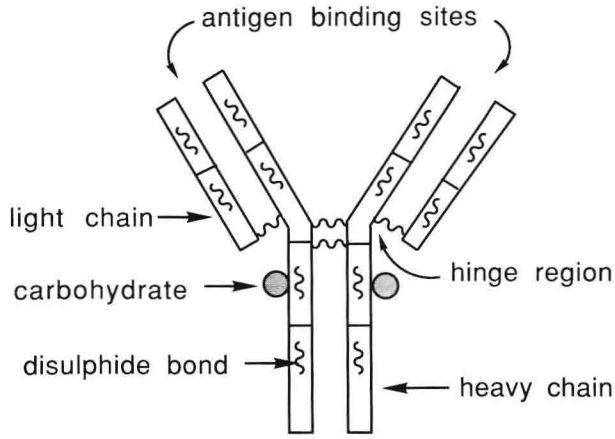


Fig. 1.1. Schematic representation of the structure of an IgG antibody molecule. It consists essentially of four protein chains, linked together by disulphide bonds. There are two heavy (H) and two light (L) chains. There are two sites which recognise antigen and these involve sites at the ends of both the heavy and light chains.

the lesion, but there was simultaneous production of other antibodies against a multitude of other normal tissue antigens so that the resulting antiserum (comprising polyclonal antibodies) would react with many normal tissues. Although it is possible to separate specific antibodies from such mixtures by the use of immunoabsorbents, this requires the availability of the target antigen in pure form. Such purified antigens were generally not available, and often the very objective of this sort of immunisation was to see whether such antigens even existed.

Theoretically, if single lymphocytes or plasma cells could be separated from animals producing polyclonal antiserum and grown in isolation, each of these clones would be producing a single species of antibody, or monoclonal antibody. These antibodies could be tested for specific reaction only against the target lesion and could be available for *in vivo* investigation. The fundamental problem in this case is that such antibody-producing cells cannot be maintained *in vitro*. The breakthrough in this area came in the 1970s when Kohler and Milstein (1975) recognised that malignant forms of antibody-producing cells, myeloma cells, can survive virtually indefinitely in *in vitro* culture and can continue to produce immunoglobulin. They showed that using recombinant genetics it was possible to hybridise such a myeloma cell with a B lymphocyte to construct hybrid cells (hybridomas) which secrete antibody determined by the B lymphocyte and which are virtually immortal due to the malignant nature of the myeloma.

The now standard hybridoma technology is to immunise an animal, usually a mouse (but sometimes a rat) with the antigen or target tissue such as tumour cells, and to fuse lymphocytes prepared from its spleen (rich in B lymphocytes) with a specially selected and cultured myeloma line from the same strain of animal (Fig. 1.2.). The myeloma lines used are those that do not normally secrete their own immunoglobulin, so that only the antibody dictated by the lymphocytes will be secreted. There are a number of techniques for encouraging cells to fuse together, but fusion is most usually induced by the presence of polyethylene glycol in the tissue culture medium.

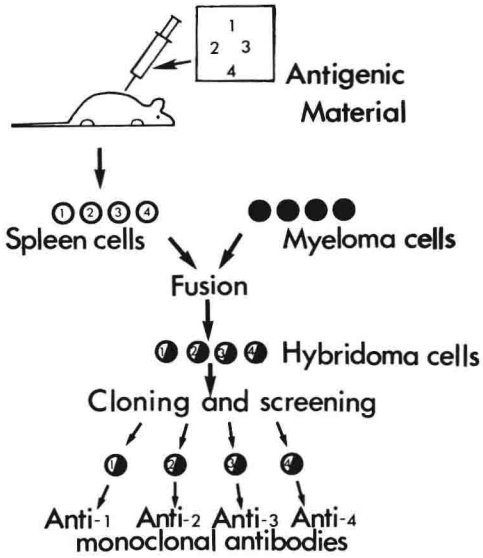


Fig. 1.2. Production of a monoclonal antibody. Animals, usually mice, are immunised with the antigenic material. Lymphocytes, generally taken from the spleen, are fused with myeloma cells to give hybridoma cells. These are then cloned to give a number of hybridomas. Each of the hybridomas generated will be producing monoclonal antibody against a different antigen present within the original immunising material or against different determinants on the same antigen. The clones therefore need screening for production of antibody against the particular antigen of interest.

Following fusion, unfused B lymphocytes are not able to survive in culture and die out. The cell population would therefore consist of the progeny of the hybrid cells, producing antibodies, and the progeny of any unfused myeloma cells. To prevent the cell population becoming overgrown with myeloma cells, an added step in the handling of the fused cells is to add a mixture of hypoxanthine, aminopterin, and thymidine (HAT) to the culture medium, which is toxic to the myeloma cells but not to hybrid cells (where resistance to this mixture is conferred by metabolic characteristics derived from the spleen cell). Thus the only cells which can survive continued culture are the hybrid cells.

Following fusion and selection, the mass cultures consist of a mixture of different hybridoma types producing antibodies against different antigens or different epitopes of the same antigen. The culture medium from these mass cultures can be tested at this stage to find out whether antibodies reactive with the antigen or specifically with the tissue of interest are being produced. The next part of the process towards monoclonal antibody production is to clone the hybridoma cells. This is done usually by a process of limiting dilution in which cell suspensions are diluted so much that the small volume which is to be inoculated into wells of microculture plates will contain on average only one cell. Following incubation, the wells containing growth are selected and their medium tested for antibody against the antigen. Cells from positive wells are grown up further, and usually cloned at least one more time before they are regarded as monoclonal and their antibodies as monoclonal antibodies.

One of the great advantages of this technology is that as well as generating monoclonal antibodies against known antigens, it can also be used to search for other as yet unidentified antigens. For example, if mice were originally immunised with tumour tissues, clones can be tested for production of antibody reactive with that type of tumour (for example, colon carcinoma) but not with the corresponding normal tissue (for example, normal colonic tissue). Many of the human tumour-associated antigens which are of interest for tumour imaging and in the understanding of tumour structural biochemistry have been identified in this way (Reisfeld and Cheresh, 1987). Thus monoclonal antibodies are useful not only in immunoscintigraphy directed against specific antigens, but also in their isolation and characterisation. This ability to isolate the antigen identified by the monoclonal antibody means that purified antigen can then be used to immunise animals to generate further antibodies against the same antigen. These "second

generation'' antibodies may have potential advantages over the original antibody in a number of ways, such as different avidity or different isotypes with better fragmentation characteristics (see below).

Although these monoclonal antibody-defined antigens associated with tumours are antigens in the sense that they are identified by antibodies, it should be recognised that they are most likely not antigenic in patients and that patients are not producing any immune response to them. To call them simply *tumour antigens* is a misnomer which can lead to some confusion.

Hybridoma technology as outlined above is now a fairly routine technique in immunology, but is nevertheless a lengthy procedure. Animals which are to provide spleen cells for fusions are generally immunised several times with antigenic material, over a period of several weeks or months. Subsequent fusion, screening, cloning, recloning, and testing of hybridomas takes many more weeks. Once cloned and established, hybridomas can be cultured in conventional tissue culture systems. Theoretically, hybridoma cells will grow indefinitely and continue to produce monoclonal antibody. In practice it is sometimes found that clones are unstable and will start to produce only low levels of antibody or sometimes stop antibody production altogether. Thus it is important to preserve seed lots of each hybridoma as soon as it has been established as monoclonal and shown to be producing antibody of interest. These seed lots can be used periodically to initiate new cultures of the hybridoma.

Hybridomas secreting monoclonal antibodies can be grown *in vitro* in tissue culture, and the spent medium from the cultures used for purification of the antibody. Large scale *in vitro* culture is expensive because of the cost of the medium and particularly because of the cost of serum, usually foetal calf serum, which is essential for cell growth and has to be used as a medium supplement. Recently, suitable non-serum protein supplements have been introduced, and this means that large-scale growth of hybridomas is becoming cheaper. Hybridomas can be grown in static flask culture, in roller bottles or, as is now becoming more common, in suspension culture in long-term, fermenter-type culture vessels with continual input of fresh medium and tap-off of antibody-rich medium.

At one time an alternative to *in vitro* growth was to grow the hybridoma cells *in vivo*. Generally the original myeloma cell line used for the fusion is from the same strain of mice which is immunised with the antigen to provide spleen cells. Thus the resulting hybridoma can grow in that strain of mice, with the growth potential coming from the malignant nature of the myeloma. The usual way to grow the hybridoma is as an ascites by injecting hybridoma cells intraperitoneally. Mice would eventually have to be killed as they became moribund with the load of ascites tumour. Ascitic fluid and serum from these mice are rich in monoclonal antibody which has continued to be secreted by the hybridoma. This *in vivo* method to produce ascites grown antibody was widely used in early work to produce antibody for immunoscintigraphy but has now generally been superseded by *in vitro* culture. The *in vivo* method has the disadvantages that prolonged *in vivo* exposure of the antibody can lead to some loss of antigen-binding activity, purified antibody can be contaminated with normal immunoglobulins from the mouse, and viral and other microbial contamination of the purified antibody is more likely from mice than it is from sterile *in vitro* techniques.

Human monoclonal antibodies. Most monoclonal antibodies currently available for immunoscintigraphy are of