TOPICS IN PHARMACEUTICAL SCIENCES 1985

D.D. BREIMER AND P. SPEISER EDITORS

ELSEVIER

TOPICS IN PHARMACEUTICAL SCIENCES 1985

Proceedings of the 45th International Congress of Pharmaceutical Sciences of F.I.P., held in Montreal, Canada, September 2-6, 1985

Editors

D.D. BREIMER

Scientific Secretary of F.I.P., Secretary of the Board of Pharmaceutical Sciences of F.I.P., Professor of Pharmacology, Center for Bio-Pharmaceutical Sciences, University of Leiden, The Netherlands

P. SPEISER

President of the Board of Pharmaceutical Sciences of F.I.P., Professor of Pharmacy, ETH-Zürich, Switzerland

1985

ELSEVIER SCIENCE PUBLISHERS
AMSTERDAM · NEW YORK · OXFORD

ISBN 0 444 80749 7

Published by:
Elsevier Science Publishers B.V. (Biomedical Division)
P.O. Box 211
1000 AE Amsterdam
The Netherlands

Sole distributors for the USA and Canada:
Elsevier Science Publishing Company Inc.
52 Vanderbilt Avenue
New York, NY 10017
USA

Library of Congress Cataloging-in-Publication Data

International Congress of Pharmaceutical Sciences of F.I.P. (45th : 1985 : Montréal, Québec)
Topics in pharmaceutical sciences, 1985.

Bibliography: p.
Includes index.
1. Pharmacy--Congresses. 2. Pharmaceutical technology --Congresses. I. Breimer, Douwe D., 1943- II. Speiser, Peter, 1921- . III. Title.
RS3.I59 1985 615'.1 85-27479
ISBN 0-444-80749-7

Printed in The Netherlands

¥516.25元

PREFACE

Topics in Pharmaceutical Sciences 1985: the title of this volume clearly illustrates the continuity of this series as published every other year since 1981. It contains the invited papers presented at the 45th International Congress of Pharmaceutical Sciences of the Fédération Internationale Pharmaceutique (F.I.P.; International Pharmaceutical Federation) held in Montreal, Canada, 2-6 September 1985. There were 9 symposia with 4 speakers each and in addition 3 up-date lectures and 272 poster and oral scientific communications were presented. The total number of participants in the Congress was almost 2000.

The topics of the nine symposia were selected by the Board of Pharmaceutical Sciences of F.I.P. in association with coordinators in the different fields (names mentioned with the symposia in this volume). This year the emphasis was on the multidisciplinary aspects of various topics in the pharmaceutical sciences and not primarily associated with single disciplines, which makes the contents of this volume quite unique and clearly illustrates that a multidisciplinary approach in pharmaceutical sciences offers most interesting research opportunities with major implications for improved understanding of drug action and drug therapy.

We are grateful to the invited speakers for conforming to the deadline of delivering their manuscripts so promptly. This made it possible to publish these Proceedings shortly after the Congress.

D.D. Breimer and P. Speiser.

CONTENTS

THE USE OF MONOCLONAL ANTIBODIES IN PHARMACEUTICAL SCIENCES

Co-ordinator and chairman: L.Z. Benet, San Francisco, USA

Topics in Pharmaceutical Sciences 1985
D.D. Breimer and P. Speiser editors

MONOCLONAL ANTIBODIES IN THE PHARMACEUTICAL SCIENCES

Bobbe L. Ferraiolo and Leslie Z. Benet
Department of Pharmacy, University of California, San Francisco, CA 94143 (USA)

INTRODUCTION

The development of methods to easily produce large quantities of homogeneous monoclonal antibodies (MCAs) has generated great enthusiasm about their potential to serve as therapeutic aids and agents. This potential has been (partially) realized for certain applications of MCAs: there have been encouraging results in renal transplantation, prevention of graft versus host disease and therapeutic drug monitoring. Implementation of other applications of MCAs has been fraught with problems associated with the MCAs themselves, their targets and the response of the recipient.

Before proceeding to the discussion of the applications and limitations of MCAs, a brief introduction to the structure and function of antibodies and a definition of terms is necessary. The general structure of an antibody molecule and its important fragments is shown in Fig. 1. As a result of their antigen binding capabilities, antibodies may neutralize bacterial toxins and viruses, they may enhance the phagocytosis of bacteria (opsonization), or they may be cytotoxic through complement fixation or other mechanisms. In addition, antibodies may perform various controlling and regulatory functions which are class and subclass dependent; they may stimulate or direct the activity of effector cells (1,2) and provide an interface between cellular and humoral immunity (3). Antibody fragments ($F(ab')_2$, Fab, Fv; Fig. 1) may be produced by enzymatic digestion of the antibody with pepsin, papain (3) and trypsin plus papain (4), respectively. These fragments retain their antigen binding function, but lose the ability to opsonize, arm effector cells or fix complement (1).

The term "monoclonal antibody" refers to a single antibody of one specificity (idiotype) and one heavy and light chain type produced by a single antibody producing cell which has been immortalized by fusion with a myeloma cell to form a hybridoma. The cloned progeny provide (indefinitely) large amounts of a single monoclonal antibody (5). Mouse, rat and recently, human (6) MCAs have been produced by this technique (3).

Some of the potential and current applications of MCAs (or MCA fragments) in the pharmaceutical sciences are listed in Table I. This review is primarily a discussion of current applications of monoclonal antibodies in human studies in vivo (where such studies exist). Space limitations preclude the discussion of

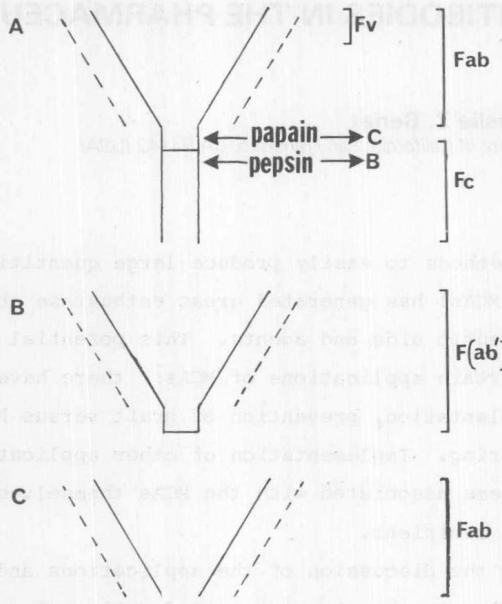

Fig. 1. A: Schematic representation of an antibody molecule; solid lines = heavy chains, dashed lines = light chains, Fv = variable regions of heavy and light chains (antigen binding site). B: Digestion of antibody with pepsin results in $F(ab')_2$ fragment. C: Digestion of antibody with papain results in two separate Fab fragments.

all aspects of MCAs and their applications in depth; many additional studies have employed polyclonal antisera and/or have been conducted in animals or in vitro. The interested reader is directed to the references in Table I and previous reviews (general: 3,7-12; cancer: 2,13-18; imaging: 19-21; infections: 22,23) for further information.

APPLICATIONS OF MONOCLONAL ANTIBODIES: CANCER

A great deal of optimism accompanied the initial suggestion of MCAs as therapeutic agents for the treatment of cancer in humans. Although the actual mechanism of tumor cell killing by MCAs in vivo is not known, some of the possibilities include: stimulation of host cellular immune responses to the tumor;

Table I

MONOCLONAL ANTIBODIES IN THE PHARMACEUTICAL SCIENCES

Applications	References
Cancer	
Treatment	24,25,27-34
Autologous Bone Marrow Transplant	47-49
Transplantation	
Bone Marrow (GVHD)	47,53-60
Kidney	62,65,68-78
Therapeutic Drug Monitoring	79-85
Drug Intoxication Reversal	35,86-88,103
Imaging	
Cancer	91-100
Infarcts	101-103
Infections	
Bacteria	104-112
Viruses	113-116
Parasites	117-121

destruction of antibody-coated tumor cells through complement-dependent lysis; or direction of phagocytosis through the reticuloendothelial system (activation of effector cells) (2,14-17). Antibody isotype (and species) may be an important determinant of the mechanism of tumor cell killing (15,16).

The overall results of clinical trials with MCAs in cancer patients have been disappointing (13,15,16). Most of the studies have been complicated by formation of antibodies to the MCA or antigenic modulation (loss of target antigen from tumor cells). A patient with refractory T-cell leukemia was given mouse MCA (anti-Leu-1) against a normal T-cell differentiation antigen (24). After treatment, circulating target antigen appeared and a transient decline in renal function ensued. A subsequent dose of the MCA was ineffective, presumably due to the circulating antigen. Antigenic modulation was also encountered. Anti-mouse-MCA antibodies were formed in this patient, but no major toxicities were associated with the treatment. Patients with acute lymphoblastic leukemia were treated with an MCA (J-5) specific for common acute lymphoblastic leukemia antigen (CALLA) with minimal toxicity, but a persistent therapeutic response was not achieved (25). Resistance to MCA treatment developed in these patients and the authors suggest that antigenic modulation was responsible (14,26). There was a striking loss of the target antigen from the surface of the leukemic cells; the cells re-expressed CALLA after 8 days. An anti-T-cell MCA (T101) was used to treat patients with chronic lymphocytic leukemia (27,28). Results in these

studies were not dramatic, and antigenic modulation (28) as well as systemic reactions to the MCA infusions were reported.

A patient with advanced cutaneous T-cell lymphoma was treated seventeen times with a mouse anti-T-cell MCA (anti-Leu-1) without toxic symptoms (29). Although complete remission was not achieved, the clinical response was good in skin, lymph nodes and blood. No anti-mouse-MCA immune response was observed; antigenic modulation occurred after each treatment but resolved in a few days. A second study in patients with cutaneous T-cell lymphoma used MCA T101 (30). Clinical responses were good in 4/6 patients but lasted only a few weeks. Anti-mouse-MCA antibodies appeared, and antigenic modulation as well as nonspecific toxicity were observed. An MCA directed against a lymphoma-associated antigen was administered to a patient with diffuse, poorly differentiated lymphocytic lymphoma (31). Circulating tumor antigen was a problem in this study and tumor cells were only transiently cleared from the circulation. Some nonspecific toxic responses to MCA administration were reported. Anti-Leu-1 MCA was used to treat patients with T-cell lymphomas (32). Five (of seven) patients showed tumor responses, but these were of short duration and four patients developed anti-mouse-MCA antibodies.

There is one report of successful treatment of a hematological malignancy with MCAs. An anti-idiotype MCA was used to treat a patient with advanced B-cell lymphoma with dramatic results (33), in spite of the presence of free target antigen in the patient's serum. No toxicity associated with administration of the MCA was observed. The patient remained in remission for more than 15 months follow-up. This type of treatment, however, requires MCAs "tailor-made" to the idiotype of the antibody present on the patient's tumor cells.

There have been relatively few studies with MCAs in the treatment of solid, nonhematological malignancies. One clinical trial employed MCAs in patients with gastrointestinal tumors (34). There appeared to be some reduction in tumor size in one patient, but it was difficult to relate this response to tumor cell lysis by the MCA. Three of the four patients developed anti-mouse-MCA antibodies, and there was some evidence of antigenic modulation by the target cells.

It is apparent that cancer therapy in vivo with MCAs has shown only limited success as yet. The pitfalls or limitations of in vivo therapy with MCAs are listed in Table II with some possible solutions. Immunogenicity of the MCA has been a problem in many of the studies in humans (14,17). The recipient may develop anti-mouse-MCA antibodies (murine MCAs have been used in almost every case) that intercept the MCA before it reaches its target, and the immune complexes formed may result in toxic side effects. The use of human MCAs (or their fragments, which are reported to be less immunogenic (35)) may circumvent this problem, though anti-idiotype responses may occur (36). Another major

Table II

LIMITATIONS OF MONOCLONAL ANTIBODIES

Problem	Possible Solution
Immunogenicity	Immunosuppress before treatment
	Induce tolerance to foreign MCA
	Use human MCAs
	Use MCA fragments
Specificity for Antigen	Use anti-idiotype MCA
Tumor Cell Escape	
Inaccessible	Intravascular or diffuse disease
	Prereduce tumor load
	Early treatment
	Use MCA fragments
Antigen Expression	
Heterogeneous Antigens	Use more than one MCA
Antigen-Negative Mutants	Use more than one MCA
Change or Lose Antigen	Cell viability antigen
	Use MCA-toxin conjugate
	Use univalent MCA or fragments
	In vitro therapy (bone marrow)
Circulating Antigen	Plasmapheresis before treatment
	In vitro therapy (bone marrow)
Ineffective In Vivo	Use human MCAs
	Schedule or route of administration
	Augment host effector mechanisms
	Use MCA fragments
	Use toxin or drug conjugates
Nonspecific Toxicity	Use MCA fragments

problem in tumor therapy with MCAs is cross-reactivity with normal tissue (13); as yet no truly tumor-specific antigens have been found (except anti-idiotype MCAs for B-cell tumors) (16). The seriousness of this limitation, of course, depends upon the extent of cross-reaction and the function of the normal cells affected.

Tumor cells may escape MCAs because they are inaccessible; MCAs face the same penetration problems (perhaps intensified because of their larger size) as conventional drugs. Solid or poorly vascularized tumors may be resistant to MCA therapy. In addition, the target antigen may not be expressed on all tumor cells (13,36-39), or MCA therapy may select for antigen-negative mutants (16). The use of mixtures of MCAs may address this problem. The tumor cells may transiently lose or change their surface antigens (antigenic or immune modulation) in response to MCA therapy (14,16,26,40,41). Univalent antibodies (42,43) or Fab fragments, which cannot cross-link surface antigens, have been suggested as

solutions to this problem. Target antigen that is shed or secreted into the circulation may intercept the MCA before it reaches its target and interfere with MCA therapy (14,17). Finally, MCAs that are very effective *in vitro* may be inactive *in vivo*; host effector mechanisms which are necessary to remove MCA-coated tumor cells (44-46) may be depleted (16).

Autologous Bone Marrow Transplantation

The use of MCAs for *in vitro* treatment of bone marrow to remove malignant cells prior to autologous transplantation avoids many of the problems associated with serotherapy. This approach involves removal of the patient's bone marrow prior to systemic chemo- or radiotherapy. The marrow is treated *in vitro* to remove residual tumor cells and re-infused after the patient receives supra-lethal therapy (8,15,16,47). Circulating antigen is removed with plasma and replaced with tissue culture medium; antigenic modulation may be minimized depending upon the length and temperature of incubation; immunogenicity of the MCA is not a factor since it is removed before bone marrow is administered to the patient; and multiple treatments may be performed and complement may be added to optimize target cell removal (16,47).

Malignant cells have been eliminated from human bone marrow *in vitro* with MCA (J-5, anti-CALLA) before autologous transplantation in patients with acute lymphoblastic leukemia (48). The patients received ablative chemotherapy and total body irradiation before their treated bone marrow was re-infused. Engraftment was successful in all patients; two patients continued in remission for more than one year. This technique has also been used in one patient to remove malignant neuroblastoma cells from bone marrow (49) in a study using MCAs conjugated to magnetic microspheres (see also 2). Previous *in vitro* studies with human bone marrow have shown that MCAs (50) and MCA-ricin toxin A chain conjugates (51,52) will eliminate malignant cells (human lymphoma and leukemic T cells) without injuring normal bone marrow cells. Longer follow up and larger numbers of patients are required before the results of the clinical studies described above may be critically evaluated (see also 47). It has been suggested that recurrence of disease in the clinical trials was probably due to failure to eradicate the tumor in the patient and not due to residual tumor cells in the marrow (8,47).

TRANSPLANTATION

Bone Marrow Allografts

One of the serious (and often fatal) consequences of allogeneic bone marrow transplantation is the occurrence of graft versus host disease (GVHD). Immunocompetent T cells in the donor marrow are believed to be responsible; they

attack the immune-compromised recipient, causing GVHD. MCAs may be useful to prevent GVHD after bone marrow allografts. The donor marrow is purged of immunocompetent T cells *in vitro* with MCAs; this treatment does not affect the marrow's capacity to reconstitute the patient's immunological function (7,8,47).

Several clinical trials in humans have illustrated the efficacy of monoclonal antibodies in the prevention of GVHD. In a study in one patient (53) with severe combined immunodeficiency (SCID), HLA-mismatched donor bone marrow was treated with an anti-T-cell MCA (anti-T12) and complement to remove mature T cells. After engraftment, GVHD developed, but was terminated by i.v. anti-T12 MCA (5 days). Immune reconstitution was successfully maintained for more than one year with no further GVHD; a state of tolerance developed to host histocompatability antigens. Two other patients (one with SCID and another with osteopetrosis) have received haplotype mismatched bone marrow treated with anti-T12. Both patients have been successfully reconstituted without GVHD (47). In two studies (54,55), pretreatment of donor marrow with MCA OKT3 (anti-T-cell) decreased, but did not completely prevent, severe GVHD. Results with OKT3 were not dramatically improved when complement was added to the donor marrow incubation (56).

Several studies have investigated the ability of MCAs to prevent GVHD in recipients with HLA-matched donors. Mixtures of anti-T-cell MCAs have been tested in some of these studies. With MCA MBG6 (anti-T-cell), MCA RFT8 (anti-T8+) plus complement (57), no patients developed GVHD greater than grade II; with MCA OKT3, MCA OKT11 plus complement (58), no acute GVHD was observed.

MCA-ricin toxin conjugates have also been used in the prevention of GVHD. Clinical trials (59,60) employed a cocktail of anti-T-cell immunotoxins (MCAs TA-1, UCHT-1, T101 conjugated to intact ricin). Engraftment of the donor marrow was successful, but in one study (59) two out of the fourteen patients treated developed severe GVHD. No toxicities developed that could be related to the *ex vivo* toxin treatment. These authors present evidence in an *in vitro* study (61) that MCA-ricin conjugates are more effective in target cell killing than MCA-toxin A chain conjugates.

Although the results of the clinical studies involving MCA-mediated prevention of GVHD have been positive overall, the number of patients investigated is small. The optimal method for prevention of GVHD with MCAs remains to be defined (47).

Renal Allografts

MCAs may be useful in the future for the prediction of rejection episodes and responsiveness to therapy in renal allograft recipients. Variations in lympho-

cyte subpopulations in allograft recipients may be related to graft survival and response to therapy. These variations can be monitored with MCAs to lymphocyte surface antigens (8,62-67), though the interpretation of these ratios is controversial.

MCAs have also been used in the treatment or prevention of acute renal allograft rejection. A number of clinical studies have employed the mouse anti-T-cell MCA, OKT3. The results in all of these trials (62,65,68-72) were similar: 84-100% reversal of graft rejection (100% reversal in 5/7 studies); subsequent rejection episodes were common (63-88%); one-year survival of grafts was good (60-89%). In all of these studies nearly every patient treated with OKT3 developed anti-mouse-MCA antibodies which precluded treatment of subsequent rejection episodes with OKT3. One study (62) also reported target antigen loss from the cells in response to OKT3 therapy. Toxicities included febrile reactions, bronchospasm and hypotension, but these reactions usually occurred only after the first administration of MCA. A recent randomized, multicenter trial with OKT3 in 123 patients undergoing acute rejection showed dramatic results: 94% rejection reversal and improved one year graft survival (73). The MCA OKT3 was also used prophylactically in one study of renal allograft recipients (74). Antigenic modulation was observed in the patients, but rejection did not result.

Similar results were reported in clinical trials with an antiblast MCA CBL1 (75,76) and an MCA against human mononuclear leukocytes (CHAL1) (77). These studies claim fewer subsequent rejection episodes with these MCAs, but most of the patients developed anti-mouse-MCA antibodies (75) or anaphylactic reactions. MCA anti-T12 has also been used in 19 patients suffering acute rejection episodes (78). Seven patients responded well, four were equivocal and eight did not respond. Eleven patients maintained their grafts (1-15 months follow up) with only one rejection recurrence. One case of allergic reaction was reported and 8/14 patients developed anti-mouse-MCA antibodies. In addition, a T12 negative population of lymphocytes emerged after 1-2 days of MCA therapy in spite of the fact that anti-T12 does not cause antigenic modulation in vitro.

The results of clinical trials using MCA's for reversal of acute renal transplant rejection are highly encouraging, in spite of the relatively few MCA specificities that have been tested. The most promising MCA tested is OKT3 which has been shown to be superior to conventional steroid treatment (73) in reversing rejection (94% versus 75%) and in improving graft survival rates (62% versus 45%). Combining OKT3 with other MCAs (CBL1, T12, CHAL1) that exhibit lower rejection recurrence rates may be one approach to optimization of therapy in the renal transplant population.