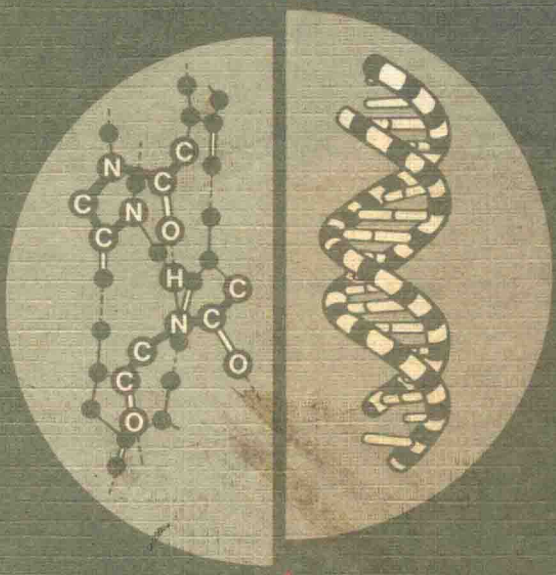
Editor: Yu.A. Ovchinnikov

PART C

PROCEEDINGS OF THE 16TH FEBS CONGRESS



Molecular bases of immune reactions

Genetic engineering

Biotechnology

Structure and function of nucleic acids

Bioorganic chemistry of low molecular bioregulators

Theoretical biochemistry

VNU SCIENCE PRESS | UTRECHT, THE NETHERLANDS

Proceedings of the 16th FEBS CONGRESS

Part C

25–30 June 1984, Moscow, USSR

Edited by
YU. A. OVCHINNIKOV

VNU SCIENCE PRESS UTRECHT, THE NETHERLANDS

VNU Science Press BV
P.O. Box 2073
3500 GB Utrecht
The Netherlands

First published 1985

ISBN 90-6764-047-6 Part C
ISBN 90-6764-044-1 set of three parts

Printed in Great Britain by J. W. Arrowsmith Ltd, Bristol

PREFACE

The investigation of the chemical nature of cell processes plays a key role in the study of living matter; the present advances in biochemistry, bioorganic chemistry, molecular biology, genetic engineering, etc. are widely known. Practically every day we are witnessing the revelation of new facts, the discovery of new bioregulators and the deciphering of new structures. The new direction in science, which is often called physico-chemical biology, not only strikes our imagination, but also has a considerable influence on the improvement of health care, efficiency in agricultural production and the development of new technologies.

In the summer of 1984, Moscow was the venue of the 16th Meeting of the Federation of European Biochemical Societies (FEBS). More than 4000 participants gathered in Moscow; this included not only Europeans, but also researchers from America, Asia and other parts of the world.

The scientific programme of the 16th FEBS meeting was very wide and covered practically all major aspects of the study of living matter on a molecular level. The lectures and posters presented at the meeting were devoted to the structure and function of biopolymers, the questions of the cell and membrane biology, the pressing problems of immunology, enzymology, neurobiology and modern directions of biotechnology.

The scientific level of all symposia organized within the framework of the meeting was extremely high and has reflected the latest achievments in each particular branch of science.

This three-part publication of the Proceedings of the 16th FEBS Congress includes the lectures that are of particular interest. We unfortunately could not publish all of the contributions—this would be hardly practicable.

On behalf of the organizing committee I should like to express my sincere gratitude to all attendants of the meeting for their active participation in this outstanding biochemical forum. I hope that the spirit of cooperation, mutual understanding and friendship which has marked the Moscow FEBS meeting will contribute to future progress in the study of living matter, to the well being of all nations and to peace and happiness on Earth.

Professor Yu. A. Ovchinnikov
Moscow

ORGANIZING COMMITTEE

Chairman	Professor Yu. A. Ovchinnikov
Vice-chairman	Professor S. E. Severin
Secretary-general	Professor V. F. Bystrov
Members	

I. G. Atabekov	P. G. Kostyuk
A. A. Bayev	A. A. Krasnovsky
I. V. Berezin	E. M. Kreps
A. E. Braunstein	W. L. Kretovich
E. I. Chazov	R. V. Petrov
V. A. Engelhardt	E. S. Severin
G. P. Georgiev	V. P. Skulachev
G. R. Ivanitsky	A. S. Spirin
V. T. Ivanov	I. V. Torgov
M. N. Kolosov	Yu. M. Vasiliev

CONTENTS

Symposium XX
Bioorganic chemistry of low molecular bioregulators

Symposium XXI
Theoretical biochemistry

Symposium XVI

MOLECULAR BASIS OF IMMUNE REACTIONS

EFFECT OF THE STRUCTURE OF N-(2-HYDROXYPROPYL)-METHACRYLAMIDE COPOLYMERS ON THE IMMUNE RESPONSE; GENETIC CONTROL IN INBRED STRAINS OF MICE

B. RÍHOVÁ
Institute of Microbiology, Czechoslovakian Academy of Sciences, CS-142 20 Prague 4, Czechoslovakia.

The homopolymer of N-(2-hydroxypropyl)methacrylamide (HPMA) and copolymers of HPMA differing in oligopeptide side chains (-Gly-Gly-OH; -Acap-Phe-OH; -Acap--Leu-HMDA and -Gly-Phe-Tyr-OH) or in their content (1, 3.5 and 8.4 % mole of -Gly-Gly-OH side chains; Fig. 1), which are considered as candidates for targetable carriers of drugs (1, 2), were investigated with respect to their ability to induce antibody formation in five inbred strains of mice (A/J; Balb/c; C3H/DiScSn; C57L/J and C57BL/10ScSn) (3, 4).

The antibody response was detected by ELISA, haemagglutination or by estimation of the number of plague--forming cells (PFC).

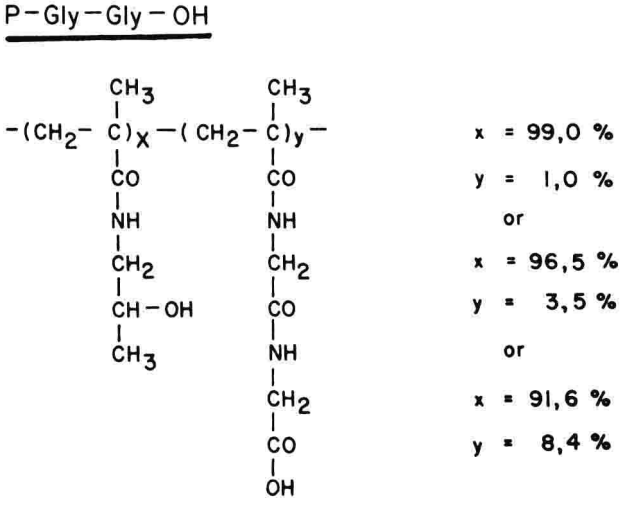


Figure 1.

Proceedings of the 16th FEBS Congress
Part C, pp. 3–7

FACTORS INFLUENCING THE IMMUNE RESPONSE TO THE HPMA COPOLYMERS

1. Composition and content of side chains

The homoppolymer poly(HPMA) was found non-immunogenic and when injected as an alum precipitate in doses in the range of 1-100 μg it did not induce a detectable level of antibodies in neither of the five inbred strains tested. The attachment of side oligopeptide sequences endowes a certain degree of immunogenicity on the copolymer molecule. The sequence -Gly-Gly-OH causes a higher immunogenicity of the copolymer than sequences -Acap-Leu-HMDA, -Acap-Phe-OH or even -Gly--Phe-Tyr-OH. The possibility that the low effect of the last copolymer is due to its high tolerogenic effect is unlikely as it was tested for immunogenicity in a wide range of doses and emulsified in complete Freund's adjuvant.

The number of side chains - in the range we have tested - does not seem to influence immunogenicity substancially. Copolymers with all three concentrations of side chains induce in mice a very similar immune response. An interesting finding is the immunogenicity of the copolymer with only 1% mole of -Gly-Gly-OH side chains which means that for every molecule of the copolymer there are only two side oligopeptide chains which represent main antigenic determinants (epitopes) (Fig. 2, detected by ELISA test).

It is possible to induce an antibody reaction against MPMA copolymers also in athymic nude mice and the antibodies, which are formed after immunization, are exclusively of the IgM isotype - it means that these copolymers behave as thymus-independent antigens.

2. Antigen dose

The mice were immunized with varying doses of copolymers from 1 to 100 μg. All copolymers were able to induce antibody formation in the whole range of doses. The dose of 100 μg led mostly to a lower antibody formation. This slightly tolerogenic effect is in full agreement with the long persistence and restricted

INFLUENCE OF SIDE OLIGOPEPTIDE CHAINS ON THE IMMUNOGENICITY OF N-(2-HYDROXYPROPYL)METHACRYLAMIDE COPOLYMERS IN DIFFERENT INBRED STRAINS OF MICE

antigen (copolymer)	(%mol) content of side chain	antibody production in inbred strains of mice*				
		C57L/J	A/J	Balb/c	B 10	C_3H/Di
		H-2^b	H-2^a	H-2^d	H-2^b	H-2^k
P^a-Gly-Gly-OH	1,0	7	8	8	9	5
P^a-Gly-Gly-OH	3,5	6	6	7	8	5
P^a-Gly-Gly-OH	8,4	7	7	5	7	4
P^a-Acap-Phe-OH	1,8	2	6	5	5	4
P^a-Gly-Phe-Tyr-OH	2,3	2	4	4	4	3
poly(HPMA)	/	no antibodies were detected				

poly(HPMA) ... homopolymer of N-(2-hydroxypropyl)methacrylamide
P^a ... HPMA copolymers with different side chains
* antibody concentration is expressed as $\log_2$ of serum dilution; titres were measured as extinction at 492 nm

Figure 2.

degradability of HPMA polymers in an organism.

3. Molecular weight of antigen

The dependance of the immune response on MW was studied with copolymer containing -Acap-Leu-HMDA side chains modified with arsanilic acid. Compared with fraction of MW 5 kD, fraction with MW between 150-200 kD increased the number of PFC releasing antibody against arsanilic acid 2-5 fold.

4. Structure of the drug (hapten)

The HPMA copolymer with -Acap-Leu-HMDA side chains was also used in a study where the molecule was modified by attachment of different chemical substances in order to mimic the situation where the copolymer is used as a drug carrier. as representatives of drugs, haptens DNP, FITC and arsanilic acid were used. The originally weak immunogenicity of the copolymer increases after attachment of the hapten groups. The overall level of antibody against the copolymer deri-

vatives depends on the hapten. The copolymers with FITC possess the highest and copolymers containing arsanilic acid the lowest immunogenicity.

5. Genetic background of the immunized organism

The ability to respond was tested in five inbred strains of mice. The C3H strain was the poorest responder to the copolymers with all three concentrations of the -Gly-Gly-OH side chain and the C57L/J strain gave the lowest response to the copolymers with -Acap--Leu-OH and -Acap-Phe-OH side chains (Fig. 2).

Greatest differences were found after immunization with the copolymer where side chains were made of glycine only, modified by arsanilic acid. In this case among all the strains tested only C3H mice responded with a pronounced response (Fig. 3).

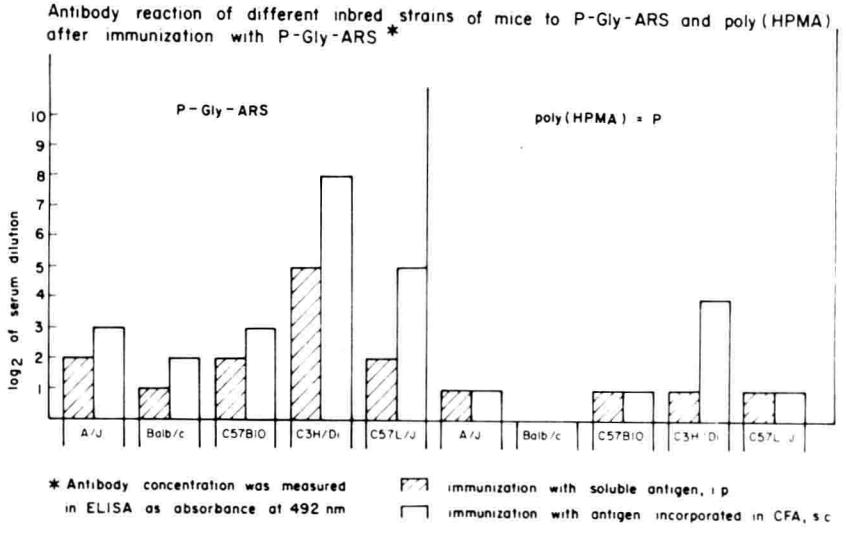


Figure 3.

ATTEMPTS OF TOLERANCE INDUCTION

Repeated injection of the non-immunogenic soluble homopolymer poly(HPMA) did not lead to induction of a state of tolerance, only after injection of 100-500 µg of the tolerogen a slight specific decrease of the immune response was observed.

REFERENCES

1. Duncan, R. and Kopecek, J. (1984). Soluble synthetic polymers as potential drug carriers. In: Advances in Polymer Sciences, Vol. 57, K. Dusek (Ed). Springer Verlag, Berlin-Heidelberg, pp. 51-101.
2. Kopecek, J. (1984). Controlled biodegradability of polymers - a key to drug delivery systems. Biomaterials 5, 19-25.
3. Říhová, B., Ulbrich, K., kopecek, J. and Mancal, P. (1983). Immunogenicity of N-(2-hydroxypropyl)methacrylamide copolymers - potential hapten or drug carriers. folia Microbiol. 28, 217-227.
4. Říhová, B. Kopecek, J. Ulbrich, K., Pospísil, M. and Mancal, P. (1984). Effect of the chemical structure of N-(2-hydroxypropyl)methacrylamide copolymers on their ability to induce antibody formation in inbred strains of mice. Biomaterials 5, 143-148.