

Immunopharmacology of Endotoxiosis

Editors

M. K. Agarwal · M. Yoshida



Gruyter

1201

63-

Immunopharmacology of Endotoxycosis

Proceedings of the
5th International Congress of Immunology
Satellite Workshop
Kyoto, Japan, August 27, 1983

Editors

M. K. Agarwal · M. Yoshida



Y077978



Walter de Gruyter · Berlin · New York 1984

Editors

M. K. Agarwal, M. Sc.; Ph. D., M. D.
Maître de Recherche au CNRS
Scientific Director: Laboratoire de Physik-Hormono-Réceptérologie
Faculté de Médecine Broussais Hôtel-Dieu
Université Pierre et Marie Curie
15, rue de l'Ecole de Médecine

F-75270 Paris Cédex 06
France

Masao Yoshida, M. D.
Professor
Department of Bacteriology
Iwate Medical University

19-1 Uchimaru, Iwate 020
Morioka
Japan

CIP-Kurztitelaufnahme der Deutschen Bibliothek

Immunopharmacology of endotoxiosis :

proceedings of the 5th Internat. Congress of Immunology
satellite workshop, Kyoto, Japan, August 27, 1983 / ed. M. K. Agarwal;
M. Yoshida. – Berlin; New York : de Gruyter, 1984

ISBN 3-11-009887-3

NE: Agarwal, Manjul K. [Hrsg.]; International Congress of Immunology
< 05, 1983, Kyoto >

Library of Congress Cataloging in Publication Data

International Congress of Immunology Satellite Workshop
(5th : 1983 : Kyoto, Japan) Immunopharmacology of endotoxiosis.

Bibliography: p.

Includes indexes.

1. Endotoxins--Physiological effect--Congresses.

2. Endotoxins--Toxicology--Congresses.

3. Immunopharmacology--Congresses. I. Agarwal, M. K.

II. Yoshida, M. (Masao), 1925- . III. Title.

[DNLM: 1. Endotoxins--immunology--congresses.

2. Endotoxins--pharmacodynamics--congresses.

QW 630 I33 1983]

QP632.E4I54 1983

616.9'2

84-7650

ISBN 3-11-009887-3

Copyright © 1984 by Walter de Gruyter & Co., Berlin 30.

All rights reserved, including those of translation into foreign languages. No part of this book may be reproduced in any form – by photoprint, microfilm or any other means nor transmitted nor translated into a machine language without written permission from the publisher. Printing: Gerike GmbH, Berlin. – Binding: Lüderitz & Bauer GmbH, Berlin. – Printed in Germany.

Immunopharmacology
of Endotoxiosis

THIS BOOK IS DEDICATED

TO

PROFESSOR L. JOE BERRY

FOR HIS CONTRIBUTIONS IN THE FIELD OF ENDOTOXINS

SPANNING OVER HALF A CENTURY

PREFACE

Bacterial endotoxins have fascinated researchers for over a century. Their beneficial effects include nonspecific increase in resistance to various sorts of infections, induction of interferon, antitumour activity, adjuvant activity, immunogenicity and radioprotection. Their pyrogenic properties had been exploited for several centuries, but this use has since been abandoned. Equally impressive is their array of noxious properties, which include the Sanarelli-Shwartzman reaction, shock, microvascular coagulation, hemodynamic alterations and the depletion of carbohydrates, to mention only a few. No organ or cell type in the host is immune from the influence of endotoxins, but it is not clear whether these effects are direct mediated and whether one site is affected or several sites simultaneously.

The purpose of this workshop was to bring together researchers from various disciplines working in the field of endotoxins. After surveying the immunopharmacological reactions evoked by the bacterial endotoxins, the influence of various pharmacological agents on endotoxin-mediated host reactions was discussed. Since the mechanism of action of these agents is sometimes quite well defined, it was hoped that insight could be gained into the manner of endotoxins reactivity in the host. This proved difficult, however, due to the diversity of experimental models. Finally, problem-oriented themes were chosen with the aim of arriving at a consensus as to the site and nature of endotoxin reaction.

It is hoped that we provided a forum for workers interested in a common problem to thrash out their differences in a con-

genial and relaxed atmosphere. If the workshop has helped re-define the problem in a clearer perspective, the goal of the organizers will have been accomplished.

The Editors

December 1983

ACKNOWLEDGEMENTS

Special thanks and appreciation are due to Professors Y. Yamamura and M. Hanaoka, President and General Secretary, respectively, of the 5th International Congress of Immunology, for their assistance regarding the opening and the publicity for this workshop.

The organizers and editors are especially indebted to Dr. M. Hirata for his painstaking and capable help in the organization and in the editorial work connected with this book.

Thanks are equally due to all the members of the Department of Bacteriology, Iwate Medical University, and to Ms. V. Braymer, Lecturer, Iwate Medical University, for their assistance during various phases.

For financial assistance we wish to thank the following:

Packard Instruments (France S.A.)

Falk Foundation e.V. (Dr. H. Falk)

Iwate Medical University (President Pr. K. Obara)

The Society for Promotion of Science of the Alumni Association of Iwate Medical University (Chairman Pr. M. Yoshida and Chief of the Academic Section Pr. S. Katsura).

Alumni Association of the Bacteriology Department, Iwate Medical University (President Pr. R. Kawana).

Members of Ichirokukai, Company of Odashima (President Mr. M. Odashima), and several other companies also contributed to the project.

Société Française d'Immunologie kindly provided some financial assistance to M.K.A. towards travel to Kyoto.

Finally, grateful appreciation is due to all those persons who contributed at different times in different ways although the space does not permit specific, individual mention.

CONTENTS

| | |
|--|-----|
| Dissociation of Tissue Localization of Endotoxin from Endotoxin Lethality George Lázár, Elizabeth Husztik, Susanna Ribárszki and Alexander Pintér | 1 |
| Sinusoidal and Parenchymal Cells as Targets of Endotoxin Effects on the Liver Riccardo Utili, Giovanni B. Gaeta, Augusto Andreana and Giuseppe Ruggiero | 11 |
| LPS-Induced Hydrogen Peroxide Release from Peritoneal Macrophages of Normal and Immunodeficient Mice Hideyuki Kato, Hideo Yaoita, Tatsuo Saito-Taki and Masayasu Nakano | 21 |
| Induction of Metallothionein in Macrophages: A Molecular Mechanism for Protection Against LPS-Mediated Autolysis Steven R. Patierno and Duane L. Peavy | 39 |
| RES Function and a Role of Plasma HDL in Endotoxin- Poisoned Mice Shuhei Sakaguchi, Hiroharu Abe and Osamu Sakaguchi | 57 |
| Neutralizing Effects of Anti-Salmonella Re Antibody on Endotoxic Bone Marrow Reactions and Induction of Procoagulant Activity Masao Yoshida, Kazuaki Kudoh, Michimasa Hirata, Katsuya Inada and Masami Ogasawara | 77 |
| Murine Immune Responses to the Salmonella Lipopolysachha- ride Regions Emilio Jirillo, Hiroshi Kiyono, Suzanne M. Michalek, Donato Fumarola and Jerry R. McGhee | 93 |
| LPS-Induced Non-Specific Resistance to Immunodeficient CBA/N Mice Against Salmonella Infection Masayasu Nakano, Kazuyasu Onozuka and Tatsuo Saito-Taki .. | 115 |
| Antitumor Action of Endotoxin in the Mouse Nanne Bloksma, Frans M. A. Hofhuis and Jan M.N. Willers .. | 133 |
| The Endotoxin Skin Reaction in Human Cancer Patients Yutaka Katayama, Nozomi Yamaguchi, Masaaki Kanou, Hideo Hayashi, Yoichi Fujita, Masami Oshima, Kenji Ogino and Masashi Kodama | 151 |

| | |
|---|-----|
| Effects of Immobilized Lipopolysachharide on the Vx2 Tumor-Bearing Rabbits Tohru Tani, Totaro Oka, Kazuyoshi Hanasawa, Yoshihiro Nakane and Masashi Kodama | 169 |
| The Activation of Complement System by Bacterial Endotoxins and its Effect on Malignant Tumor Tissues of Mice Yutaka Katayama, Nozomi Yamaguchi, Shun-ichi Yoshida, Tomoyuki Mizukuro, Masahiro Horisawa and Masashi Kodama .. | 177 |
| Elicitation of the Shwartzman Reaction by a Combination of Endotoxin and Agents which Activate the Complement System: Microvascular Events Henry Z. Movat and Clement E. Burrowes | 197 |
| Complement in Clinical and Experimental Disseminated Intravascular Coagulation Yasumasa Furukawa, Toshikazu Yoshikawa, Masashi Murakami and Motoharu Kondo | 213 |
| Effects of Steroid, Anti-Thrombotic Agents and Defibrinogenation on Endotoxin-Induced Disseminated Intravascular Coagulation in Rats Toshikazu Yoshikawa, Yasumasa Furukawa, Masashi Murakami and Motoharu Kondo | 221 |
| Serological and Immunohistochemical Analysis on the role of Complement in Endotoxin Initiated Lethality in Mice Shigenobu Matsuo, Morio Totsuka, Hiroshi Hayasaka and Kokichi Kikuchi | 235 |
| Studies on Endotoxin-Like Properties of Several Simple Polysaccharides Takeshi Mikami, Toshihiko Nagase, Shigeo Suzuki and Masuko Suzuki | 245 |
| Bone Resorbing Potential of Endotoxin and its Immunopharmacological Modulations A. Nowotny, F. Sanavi, A. M. Nowotny, E. Kovats, J. Rothmann, D. Siegler, K. Sallay and P. H. Pham | 261 |
| Catecholamine-Hyperresponse in Endotoxemia of Mice Kazuo Kuratsuka and Reiko Homma | 281 |
| Modulation of Endotoxycosis by Steroids and Diabetogenic Agents in Responder and Refractory Mice Strains M. K. Agarwal and G. Lazar | 299 |
| Pathogenesis in the Aggravation of Acute Cholangitis : its relation to the Interplay of Endotoxemia and Host Resistance Hiroshi Shimada, Gizo Nakagawara, Fumihiko Kito, Tetsuo Abe, Mamoru Kobayashi and Shuzi Tsuchiya | 315 |

| | |
|---|-----|
| Endotoxemia in Pregnancy Due to Chloramphenicol Administration Hiroshi Irie and Wataru Mori | 331 |
| General Discussion | 345 |
| Author Index | 371 |
| Subject Index | 373 |

DISSOCIATION OF TISSUE LOCALIZATION OF ENDOTOXIN FROM ENDOTOXIN LETHALITY

George Lázár, Elizabeth Husztik⁺, Susanna Ribárszki and Alexander Pintér

Institute of Pathophysiology, Institute of Medical Biology⁺, University Medical School, Szeged, Hungary

Introduction

Correlations between reticuloendothelial activity and sensitivity to endotoxin are contradictory. The following substantiate the concept that reticuloendothelial system (RES) plays a pivotal role in resistance to endotoxin:

1) Soon after the injection of endotoxin there is deep depression in the reticuloendothelial activity, which persists until death. However, in survivors, RES function recovers and goes on to hyperfunctional state. Similar pattern of reticuloendothelial response was also observed in other types of experimental shock (1).

2) The blockade of the RES with inert, nonmetabolizable, colloidal materials sensitizes to endotoxin poisoning and abolishes the tolerance to the biological effects of endotoxin, including the lethal effect (2, 3).

3) Animals previously injected with one or more sublethal doses of endotoxin, exhibit hyperfunctional RES and increased tolerance to bacterial lipopolysaccharide (LPS) (4, 5).

Other studies, however, have indicated that the relationship between host RES activity and endotoxin sensitivity is more complex. It is true that endotoxin tolerant animals have hyperfunctional RES, but the classical RES stimulants such as BCG, zymosan, *Corynebacterium parvum*, particulate glucan

do not confer resistance of experimental animals to endotoxemia, but profoundly increase host susceptibility to endotoxin (6). Particulate glucan while increasing all parameters of RES renders the LPS nonresponder, C3H/HeJ, mice nearly as responsive as conventional mice (7). Substances not affecting RES activity such as Streptozotocin, potentiates the toxic effects of LPS in experimental animals (8); depression of RES activity by methyl palmitate, renders experimental animals highly resistant to lethal and supralethal doses of endotoxins (9).

In this study, to understand the correlations between reticuloendothelial activity and sensitivity to endotoxin, the vascular clearance and tissue distribution of ^{51}Cr -labelled endotoxin and endotoxin sensitivity have been studied in mice following treatment with gadolinium chloride (10, 11), sodium polyanethol sulphonate (12) and carrageenan (13). All of these substances, although having different physicochemical properties, significantly depress reticuloendothelial function.

Materials and methods

CFLP males weighing 30-35 g were maintained on a standard laboratory diet and tap water, ad libitum.

Gadolinium chloride (K. and K. Laboratories, Plainview, New York) was dissolved in 0.85% saline at a concentration of 2 mg/ml and was injected i.v. at a dose of 1 mg/100 g body weight 24 hours before testing; kappa, lambda, or iota carrageenan (Marine Colloids, Rochland) were dissolved in boiling 0.85% saline at a concentration of 10 mg/ml, and were injected i.p. at a dose of 5 mg/100 g body weight 24 hours before testing; sodium polyanethol sulphonate (Liquoid, Hoffman-La Roche, Basel) was dissolved in 0.85% saline at a concentration of 4 mg/ml, and was injected i.v.

at a doses of 3 or 2 mg/100 g body weight 1 hour before testing.

E. coli 026:B6 lipopolysaccharide B (Difco Lab., Detroit, lot 688839) was labelled with 50 μ Ci/mg ^{51}Cr -sodium chromate (Isotope Institute of Hungarian Academy of Sciences) by the method of Braude et al. (14). For organ-uptake studies, one hour after the i.v. injection of 250 μ g ^{51}Cr -labelled endotoxin animals were killed and the radioactivities in the blood, liver, spleen, lung and bone marrow were determined in a well-type scintillation detector and the results expressed as a percentage of the injected dose.

For measurement of endotoxin sensitivity, mice were challenged i.p. with proportional doses of endotoxin (*E. coli* 026:B6 lipopolysaccharide B, Difco Lab., Detroit, lot 688839) and the number of survivors was recorded after 48 hours. The significance of differences in the mean response between treatments was determined by the t test. The Chi square test was used to determine the significance of differences between survivors after various treatments.

Results

Data in Table 1 show that animals were sensitized to LPS by pretreatment with either 2 mg (37.5% survival) or 3 mg (15% survival) polyanethol sulphonate per 100 g body weight compared to control (80% survival) treated only with LPS. Studies with ^{51}Cr -labelled LPS show that both doses of the polyanethol sulphonate caused the retention of radioactivity in the blood and reduced the hepatic uptake of the injected radioactivity. The uptake of ^{51}Cr -labelled LPS in the spleen and lung was significantly increased (Fig. 1).

The effect of carrageenan on endotoxin sensitivity and the distribution of ^{51}Cr -labelled endotoxin were very similar to those observed in mice pretreated with Liquoid. All forms

Table 1
Sensitization to LPS lethality by Liquoid

| Treatment | Living/total (48 hr) | Survival (%) | Statistics |
|-----------|-------------------------|-------------------|--------------------|
| 1. E | 28/35 | 80 | |
| 2. L | 19/20 | 95 | 2 vs 1 $p > 0.05$ |
| 3. L + E | 3/20 | 15 | 3 vs 1 $p < 0.001$ |
| 4. L | 20/20 | 100 | 4 vs 1 $p > 0.05$ |
| 5. L + E | 6/16 | 37.5 | 5 vs 1 $p < 0.01$ |

E = endotoxin, 250 μ g/10 g body weight i.p., one hr after the injection of Liquoid for lines 3 and 5; L = Liquoid, 3 mg/100 g body weight for lines 2 and 3 and 2 mg/100 g body weight for lines 4 and 5.

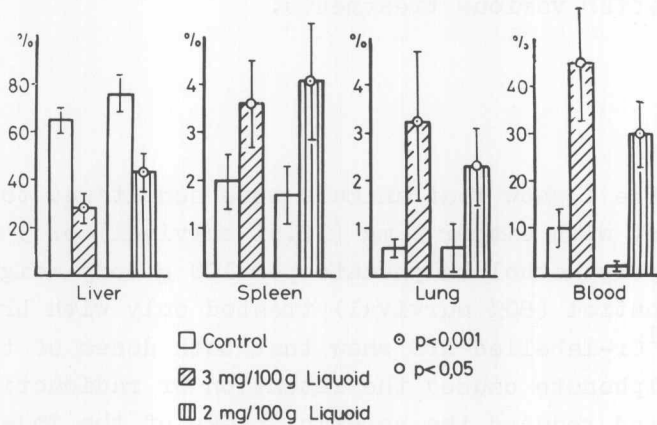


Fig. 1. Effect of Liquoid on the distribution of LPS. The radiocontents of the organs and blood were determined 1 hr after the i.v. injection of 250 μ g LPS labelled with ^{51}Cr .

Table 2.
Sensitization to LPS lethality by carrageenan

| Treatment | Living/total (48 hr) | Survival (%) | Statistics vs 1 |
|-------------|-------------------------|-------------------|--------------------|
| 1. E | 30/50 | 60 | |
| 2. Kappa C | 4/20 | 20 | p<0.01 |
| 3. Lambda C | 1/20 | 5 | p<0.001 |
| 4. Iota C | 3/20 | 15 | p<0.01 |

E = endotoxin, 250 μ g/10 g body weight i.p.; C = carrageenan, 5 mg/100 g body weight i.p. 24 hr before endotoxin administration.

of carrageenan aggravated endotoxin lethality. Only 20%, 5%, 15%, of the animals given kappa, lambda or iota carrageenans, respectively, survived endotoxin challenge compared to 60% survival in the control group given endotoxin

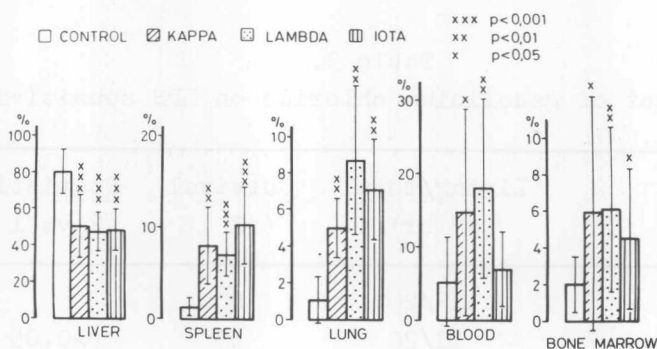


Fig. 2. Effect of carrageenan on the distribution of LPS. Carrageenan was injected i.p. at a dose of 5 mg/100 g body weight 24 hr before the i.v. injection of 250 μ g LPS labelled with ^{51}Cr . The radiocontents of the organs and blood were determined 1 hr later.

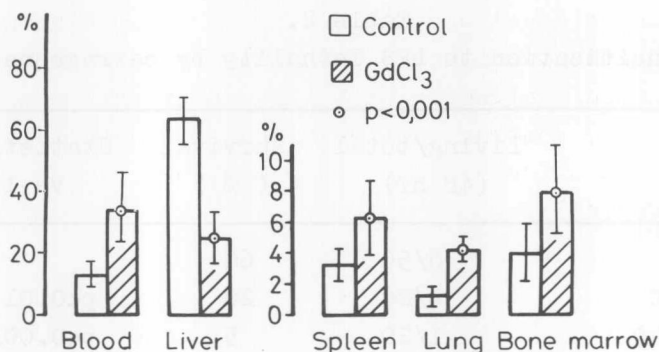


Fig. 3. Effect of gadolinium chloride on the distribution of LPS.

Gadolinium chloride was injected i.v. at a dose of 1 mg/100 g body weight 24 hr before the i.v. injection of 250 μ g LPS labelled with ^{51}Cr . The radiocontents of the organs and the blood were determined 1 hr later.

alone. Carrageenans also caused the retention of ^{51}Cr -labelled LPS in the blood and at the same time reduced the hepatic and increased the extrahepatic uptake of endotoxin (Fig. 2).

Table 3.

Effect of gadolinium chloride on LPS sensitivity

| Treatment | Living/total (48 hr) | Survival (%) | Statistics 2 vs 1 |
|-----------|-------------------------|-------------------|----------------------|
| 1. E | 12/20 | 60 | |
| 2. Gd | 11/20 | 55 | $p > 0.05$ |

E = endotoxin, 250 μ g/10 g body weight i.p.; Gd = gadolinium chloride, 1 mg/100 g body weight i.v. 24 hr before endotoxin challenge.