Immunopharmacology of Endotoxicosis

Editors
M. K. Agarwal · M. Yoshida

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

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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

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Immunopharmacology of Endotoxicosis

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, adjuvanticity, 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 efforts 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 redefine the problem in a clearer perspective, the goal of the organizers will have been accomplished.

The Editors

December 1983

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CONTENTS

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 persits 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 indicate 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

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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 µCi/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 µg ^{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 squere 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 µg/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.

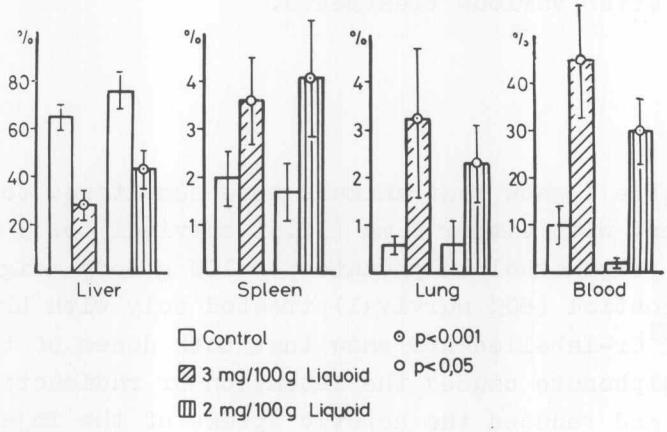

Fig. 1. Effect of Liquoid on the distribution of LPS. The radiocontents of the organs and blood were determined 1 hr after the i.v. injection of 250 µg LPS labelled with ^{51}Cr.

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 µg/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

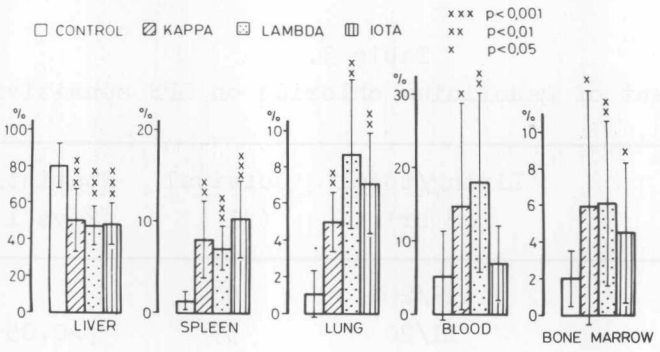

Fig. 2. Effect of carrageenan on the distribution of LPS. Carrageenan was injected i.p. at a dose of 5 mg/100 g body weight 24 hr before the i.v. injection of 250 µg LPS labelled with ^{51}Cr. The radiocontents of the organs and blood were determined 1 hr later.

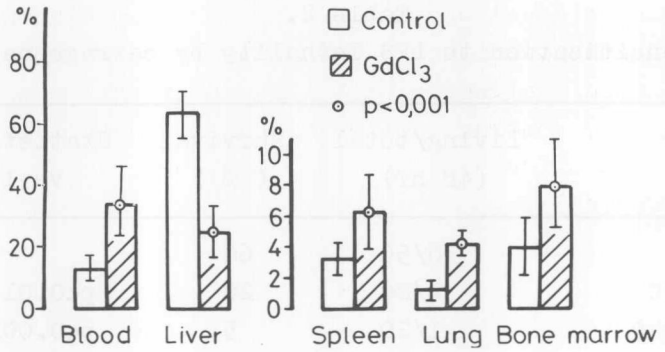

Fig. 3. Effect of gadolinium chloride on the distribution of LPS.
Gadolinium chloride was injected i.v. at a dose of 1 mg/ /100 g body weight 24 hr before the i.v. injection of 250 µg LPS labelled with ^{51}Cr. The radiocontents of the organs and the blood were determined 1 hr later.

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