Effect of RES Stimulation on Endotoxin Shock in Mice. (31312)

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The evidence for increased resistance to bacterial infections after stimulation of the reticuloendothelial system (RES) with various agents such as endotoxin from Gram-negative bacteria(1), BCG(2), zymosan(3, 4) or triolein(5) is now well established. But most of these RES stimulants render the animals increasingly susceptible to endotoxin(6, 7,8,9,10), whereas only pretreatment with endotoxin provides tolerance towards otherwise lethal doses of endotoxin. Because of its poor solubility and therefore colloidal properties, intravenously or intraperitoneally injected endotoxin is almost exclusively taken up by the RES.

At first it appears quite paradoxical that substances which induce increased phagocytosis and resistance to challenge with virulent bacteria should cause such susceptibility to one of the bacterial constituents. However, findings that increased susceptibility can be abolished by pretreatment with cortisone or can be augmented by adrenalectomy (7) emphasize that factors other than phagocytic activity of the RES and classical immune-mechanisms are concerned with resistance to endotoxin. Recent investigations on the mechanisms of endotoxin action(12) and of RES stimulation(11) suggest that both effects can be related to changes in the permeability of the lysosomal membranes of the RE cells. The point has been made(12) that tolerance to endotoxin is due to an increased stability of lysosomal membranes and its content of certain hydrolytic enzymes in the phagocytic cells of the RES. The gradual failure of the endotoxin-detoxifying mechanisms of these cells during the progression of endotoxin shock can be related to a release of their lysosomal contents(13).

Pretreatment with glucan(14), the polysaccharide fraction of zymosan, increases mortality after i.v. or i.p. injection of LD_{50} of endotoxin, whereas pretreatment with restim(15), a lipid fraction derived from shark liver, shows a protective effect. It is suggested that the contrasting findings depend on differences in lysosomes of the glucan- or restim-stimulated RE cells.

Materials and methods. A total of 524 male Swiss mice weighing 20-30 g was used in 7 experiments. They were maintained on Purina lab chow and water *ad libitum* and kept in rooms with a controlled temperature of 23°C and a humidity of 50%.

Endotoxin: lipopolysaccharide *S. typhosa* 0901 or lipopolysaccharide *E. coli* 0127:B8 (Difco Laboratories) was suspended by boiling in pyrogen-free Baxter saline and then homogenizing in a Virtis blender. The appropriate dose was injected intraperitoneally in 1 ml or intravenously in 0.2 ml of suspension. Deaths were recorded every 6 or 12 hours. The data were statistically analyzed using the 95% confidence limits of the percent mortality.

RES stimulation: glucan (Fleischmann Laboratories Lot No. IF 5500) was suspended in pyrogen-free 5% glucose solution by boiling in a water bath for 1 hour and then homogenizing. After centrifuging for 30 minutes at 4000 rpm, the supernatant was discarded and replaced by an equal volume of 5% glucose solution. Restim(15) was suspended in a 5% glucose solution. The emulsion was prepared by adding ninol (Stepan Chemical Co.), a nonionic detergent, 5% by weight of lipid, and treated for one minute at 20,000 rpm in a Virtis homogenizer. The particle size in 1.0 mg of each suspension was found to be less than 2 μ. Mice were injected intravenously with either 1.0 mg of glucan or 1.0 mg of restim 2 days prior to challenge. In carbon clearance tests it was found that repeated injections of restim did not further increase the phagocytic activity beyond that obtained by a single dose, whereas repeated glucan injections did increase the phagocytic activity markedly.

Human γ-globulin (Lederle 053-280) was injected i.p. in a dose of 0.1 ml of a 16.5% solution one day prior to challenge.

TABLE I. Modification of Endotoxin Lethality by Glucan and Restim.

Exp No.	Pre-treatment	LPS *S. typhosa*	Mortality	LD_{50} (mg)
S5	Control	.75 mg i.p.	10/20 = 50%	.75
	Restim	"	6/20 = 30%	1.25
	Glucan	"	17/20 = 85%*	.44
S6	Control	.4 mg i.p.	0/20 = 0%	—
	Restim	"	0/20 = 0%	—
	Glucan	"	6/27 = 22%*	.91

* Significant at 95% confidence level.

Control animals were injected with an equal amount of 5% glucose solution. To avoid endotoxin contamination, all glassware was thoroughly washed, autoclaved for 45 minutes and kept for at least 6 hours at 180°C. Pyrogenicity tests in male New Zealand white rabbits for glucan, restim and ninol, given intravenously in doses 4-fold higher per 100 g body weight than those used in the experiments described above, were negative.

Results. Pretreatment with glucan apparently modifies the response to endotoxin injections in a different way from that seen in restim-treated groups. The glucan-treated groups generally showed a significantly higher mortality rate than the controls, whereas restim pretreatment provided some protective action. Human γ-globulin *per se* showed no effect on mortality; however, when given together with restim, a synergistic effect was observed (Table I).

All animals were in clearly observable shock during the first 48 hours after challenge. In relation to the per cent mortality most deaths occurred in the control groups within 12 to 24 hours, in the glucan-treated groups within 6 to 24 hours, and in the restim-treated groups within 12 to 36 hours after challenge with endotoxin. The survivors started to drink and eat after 48 hours and seemed to be fully recovered by the fourth day. After 10 days, the survivors in Experiment S5 were given the same pretreatment and challenged with 1.0 mg lipopolysaccharide *S. typhosa*. They showed the anticipated lower mortality but again with the same relation to pretreatment (Fig. 1), whereas a third challenge with 1.5 mg endotoxin had no lethal effect. The average LD_{50} for i.p. injection of *S. typhosa* LPS in 30 g Swiss mice calculated from the data was:

in controls 0.85 ± 0.04 mg
after glucan, 1.0 mg i.v. 0.64 ± 0.14 mg
after restim, 1.0 mg i.v. 1.45 ± 0.16 mg

The differences in the LD_{50} of both treated groups are significant at the 95% confidence level when compared to the control.

It is now well established(16) that endotoxin administration evokes specific antibodies and it has been shown elsewhere(17) that restim increases existing levels of antibodies. To induce antibody formation 0.01 mg/mouse of endotoxin was administered 10 days prior to challenge. The combination of endotoxin and restim injections further increased tolerance to endotoxin as shown in Table II. It cannot be definitely concluded from these data whether the protective effect of restim is due to an increase in specific antibody or to a potentiation of still unknown mechanisms of endotoxin tolerance.

In agreement with the reported results are the findings of Ransom(18) that restim, when given before the intradermal preparatory dose, is able to eliminate the local Shwartzman reaction, whereas administration of glucan enhances the development of the reaction very markedly. Furthermore, experiments with burn shock in rats(19) show

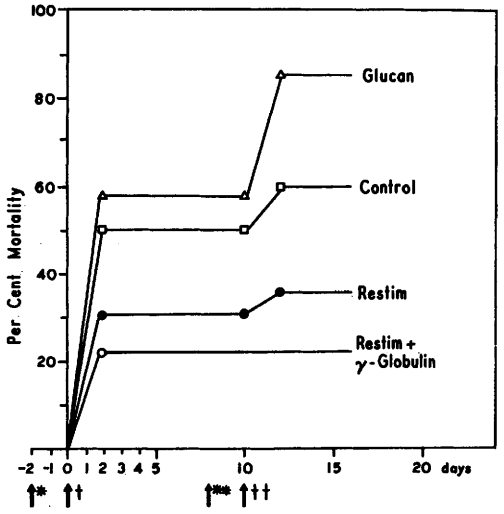

FIG. 1. Modification of endotoxin lethality by glucan and restim. * First treatment, † first challenge (0.75 mg LPS), ** second treatment, †† second challenge (1.0 mg LPS).

TABLE II. Modification of Endotoxin Lethality by Restim.

Exp No.	Pre-treatment	LPS *E. coli*	Mortality	LD_{50} (mg)
S8	Control	1.5 mg i.p.	17/17 =100%	.75
	γ-Globulin	"	20/20 =100%	.75
	Restim	"	16/20 = 80%	.94
	Restim + γ-globulin	"	15/20 = 75%*	1.00
S10	Control	1.0 mg i.v.	20/20 =100%	.50
	Restim	"	17/20 = 85%	.60
	Endotoxin .01 mg	"	15/20 = 75%*	.75
	Endotoxin + restim	"	10/20 = 50%*	1.00

* Significant at 95% confidence level.

a similar increase in susceptibility after glucan pretreatment and a decrease in mortality after restim pretreatment.

Discussion. It is generally accepted that death in endotoxin shock occurs after failure in the detoxifying capacity of various defense mechanisms and the persistence of lethal endotoxemia. High doses of endotoxin may impair the functional state of the RES either by direct damage of the RE cells(20) or by indirect action on several endocrine, neural and vascular mechanisms. The former may lead to a direct alteration of the lysosomes resulting in formation of autophagic vacuoles(21). The latter may cause tissue hypoxia and associated biochemical changes, the net result of which is an alteration in the permeability of the lysosomal membranes and consequent release of hydrolases and other potentially injurious digestive enzymes(12).

Certain parameters of the RES function such as phagocytic activity and antibody production can be increased by a number of widely different agents(1,2,3,4). Since the RES is involved in endotoxin shock—both as the site of detoxification and as a major target of the cell damaging effects of endotoxin—it appears that stimulation of the RES might prevent or reduce endotoxin shock. Phagocytic activity of RE cells assumes only a minor role in endotoxin resistance, whereas other functions of the RES appear to be more important. An obvious difference in stimulation by glucan and restim is that the former causes an excessive hypertrophy of the RES(22) while the latter apparently does not(23). Restim appears to exert its effect either by alteration of the percentage of active RE cells or by increasing the phagocytic capacity of the individual cell or both. The new hyperplastic RE cells after glucan treatment would be in a state of fast multiplication and may initially not be as competent to detoxify as the original cells. In contrast to this, the hyperfunctional RE cells in restim-treated mice appear to have an increased ability to detoxify endotoxin.

The mechanism by which endotoxin is detoxified is not yet firmly established, but it may be an enzymatic alteration of the molecule. Although it has been shown(24) that the activity of hydrolytic enzymes increases during RES hyperplasia, it is believed that hydrolytic splitting of endotoxin does not play a major role in detoxification. Increased cellular injury, however, can be expected, when membrane disruptive agents such as endotoxin are phagocytized by cells with altered lysosomal membranes. Lentz *et al*(13) showed recently that the release of lysosomal enzymes during hemorrhagic shock was twice as much in glucan-pretreated rats than in controls, in contrast to a significant lower release after zymosan pretreatment, which had a shock protective effect. It has been suggested(25) that the lipid fraction of zymosan may be involved in the development of shock protection.

The permeability of lysosomal membranes is certainly not the only parameter which is important in shock and might be altered by RES stimulants. The interference of RES stimulants with mechanisms which involve specific antibody(16), cortisone(26), glycogen metabolism(27), clotting factors(28), blood lipids(29) or other systems may influence the host's ability to resist or to detoxify endotoxin as well.

Summary. Although injected endotoxin is apparently taken up almost exclusively by the RES and death occurs after failure of the RES to remove circulating endotoxin, protection to lethal doses cannot be obtained by increasing the phagocytic activity alone. Pretreatment with glucan, which results in a marked proliferation of the RE cells, enhances the phagocytic capacity significantly,

but at the same time increases the susceptibility to endotoxin. This unequal increase in phagocytic and detoxifying capacity of the hyperplastic RE cells may be ascribed to an increased permeability of their lysosomes. Stimulation of the RES with restim increases phagocytic capacity to a similar extent, but does not result in proliferation of the RE cells. The observed protective effect of restim against endotoxin may be due to increased detoxifying mechanisms in the presence of normal or stabilized lysosomes within the hyperfunctional RE cells.

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Effects of Cobalt on Activity of Sheep Erythropoietin in Rat Kidney Homogenates.* (31313)

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Since the findings that the kidney produces an erythropoietic substance(1,2,3), several workers have attempted to extract erythropoietin from various tissue preparations. However, the reports of erythropoietic activity in kidney extracts have been controver-

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