

Review

Radio-detoxified endotoxin activates natural immunity: A review

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Abstract

It is well demonstrated that serial endotoxin injections produce endotoxin tolerance and elevate the natural immunity/resistance. However, such injections may also have harmful effects such as high fever, hypotension and abortion. For this reason endotoxin (LPS) injections are not suitable to enhance nonspecific resistance in endotoxin-sensitive species like man. Various techniques have been designed (physical, chemical, etc.) for the detoxification of endotoxins while the beneficial effects are maintained. Perhaps one of the best detoxification techniques is the treatment with ionizing radiation. The irradiation of LPS with ^{60}Co (150kGy) decreased its toxicity in a dose-dependent manner. Such radio-detoxified endotoxin (RD-LPS) preparations show decreased toxicity whereas the beneficial effects were preserved. Irradiation causes marked chemical alterations in LPS, such as a decrease of glucosamine, ketodeoxyoctonic and fatty acids. A single parenteral RD-LPS injection prevents various forms of shock in experimental animals. This preparation has a membrane-stabilizing effect, and thereby it can prevent the membrane-damaging effect of LPS and of some cytostatic agents. Unlike endotoxin, RD-LPS has little hypotensive effects, and the pretreatment with this preparation can prevent practically all the hemodynamic changes induced by LPS. LPS plays an important role in the pathogenesis of intestinal syndrome of radiation disease, which may be prevented by RD-LPS pretreatment up to 70% in rats. RD-LPS retains the adjuvant activity of LPS, and it serves as a good adjuvant for inactivated virus vaccines. RD-LPS can also evoke the regeneration of the immune system in irradiated animals. The decrease of nonspecific resistance in immunodeficient or immunosuppressed patients is the most important cause of opportunistic infections that may lead to sepsis like in endotoxaemia and pneumonia. Organ transplant recipients commonly die of septicaemia. Antilymphocyte serum (ALS) is used in such patients as an immunosuppressant. The augmentation of natural resistance and the induction of endotoxin tolerance are of major significance in such patients. In ALS-treated rats RD-LPS induces also tolerance against the lethal dose of LPS. This demonstrates that in spite of the suppressive effect of ALS on T-lymphocytes the induction of LPS tolerance (the enhancement of natural resistance) remains normal. Facultative pathogenic organisms may flourish and cause disease when specific and nonspecific resistance is impaired. RD-LPS can produce a significant proliferation of lymphoid cells in germ-free animals which are immunodeficient. Many other beneficial effects are preserved by RD-LPS preparations, such as the activation of macrophages and of the reticuloendothelial system and antitumor activity. On the basis of these favorable experimental results, RD-LPS has been tested on 350 surgical patients suffering from gastrointestinal tumors, patients suffering from acquired immunodeficiency syndrome (AIDS) and cancer patients treated with CYSPLATIN®. RD-LPS treatment prevented sepsis and activated the bone marrow function in these patients.

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1. Introduction

In the beginning of last century Wright [1] was among the first to study the natural-innate immunity/resistance. He observed that after vaccination with killed bacteria a 'negative' phase occurred, which was followed by an increased natural resistance tied to the production of specific antibodies. Similar observations were made later independently by Landy and Pillemer [2] and Rowley [3] who demonstrated that this preimmune resistance was due to the LPS content of the vaccines used. It was also observed that changes in immune status were closely related to the production of natural antibodies [4]. The properdin system, which was discovered by Pillemer et al. [5], was also stimulated by LPS. The discovery of Beeson [6] that low doses of toxic LPS given repeatedly lead to the induction of endotoxin tolerance and prevent the pyrogenic effect of LPS (pyrogen tolerance). It was also observed that small LPS doses could decrease the severity of various forms of experimental shock and of the lethal effect of radiation. Later it was demonstrated that LPS functions as an immunological adjuvant, capable of inducing tumor necrosis. It stimulates also bone marrow activity and increases the interferon production. Moreover, it was possible to prevent the development of infectious diseases by small endotoxin doses which significantly increased the natural resistance. These observations have evoked great interest in the studies on the endotoxin tolerance [7].

A question has been posed whether or not endotoxin tolerance could be used for the prevention of endotoxin shock and of shock due to other causes. During endotoxin tolerance no lysosomal membrane damage occurred even if LPS was given repeatedly [8], and the animals survived the endotoxin

challenge [9]. However, the use of endotoxin for preventive treatment has been limited by the excessive LPS sensitivity of man and higher animals. For this reason, numerous attempts (using physical, chemical and immunological approaches) have been made for the production of LPS preparations that would maintain its ability to induce tolerance, increase natural resistance and still to maintain its adjuvant property and necrotic effect on tumors, while its toxicity would be decreased or entirely eliminated. Methylation [10] and the so-called monophosphoryl lipid A [11-13] have been successful in detoxifying LPS. The capacity to induce natural immunity/resistance has been best preserved when ionizing radiation (^{60}Co -gamma) has been used for detoxification [14-17].

2. Production and biological effects of radio-detoxified LPS

E. coli bacteria are produced in bulk by fermentation and the LPS fraction is extracted by the modified phenol-water method of Westphal [14,18]. The LPS product is further purified by ultracentrifugation and dissolved in water and treated with ^{60}Co -gamma radiation using 50, 100, 150 or 200 kGy doses. The toxicity of LPS decreases in dose dependent manner [7,9,14,33].

The biological effects of radiation-treated LPS have been presented in Table 1. The harmful effects of LPS decrease after radiation, whereas its capacity to induce tolerance, to function as an immunoadjuvant, immunomodulator, to protect against shock and radiation and to stimulate natural resistance are preserved to a large extent. The lethal effect of such preparations (LD50 and LDM) depends on the amount

Table 1
Comparison of various biological effects of intact endotoxin (LPS) and radio-detoxified (5 Mrads) *E. coli* endotoxin (RD-LD) preparations

Effects	LPS	RD-LPS
LD ₅₀ (rat, mg/kg, <i>n</i> = 50)	20	50
Hypotension (dog, mmHg, <i>n</i> = 10)	<60	>10
Leukocyte decrease (rabbit, %, <i>n</i> = 10)	66	45
Platelet decrease (rabbit, %)	80	50
Fibrinogen decrease (rabbit, %, <i>n</i> = 10)	40	17
Reptilase time increase (rabbit, %)	90	10
Pyrogenicity (rabbit, °C, <i>n</i> = 10)	2.4	1.2
Sanarelli-Shwartzman local (rabbit, mm ² , <i>n</i> = 5)	1200	600
Hyperlipidemic effect (mouse, <i>n</i> = 20)	+	-
Fetopathy (rat, %, <i>n</i> = 50)	100	2
Complement depletion in vivo (rabbit, %)	35	10
Spermatozoa agglutination in vitro (swine, <i>n</i> = 50)	+	-
Anti-complementary activity in vitro (μg)	30	174
Adjuvant effect (rat, %, <i>n</i> = 100)	508	470
Interferon induction (mouse, <i>n</i> = 40)	+	+
Stem cell mobilization (CFUS/mouse, <i>n</i> = 25)	521	247
Endotoxin tolerance (rat, pig, <i>n</i> = 200; 10, protection, %)	100	100
Shock preventing (rat, dog, <i>n</i> = 250; 10, protection, %)	80	80
Radioprotective effect (rat, <i>n</i> = 300; protection, %)	70	70

of radiation applied [14,16,17]. It has been shown that the anti-complement and platelet-aggregating effect (indicating membrane damage) of detoxified endotoxin is maintained, depending on the radiation dose used (Fig. 1). The decrease of membrane perturbation by radiated LPS has been proven by the measurement of ³H-concanavalin binding [19]. The comparison of major effects of LPS and radio-detoxified endotoxin (RD-LPS) are shown in Table 1.

Toxic LPS increases the blood level of very low density lipoprotein (VLDL) in mice, whereas the RD-LPS does not. Thus hyperlipidaemia can be used as a marker of LPS toxicity [20]. RD-LPS is a lesser inhibitor of membrane-bound adenylate cyclase (AC) than its toxic counterpart [21]. Depending on the radiation dose used, RD-LPS preparations do not release or show a decreased release of lysosomal enzymes (beta-glucuronidase or cathepsin D). The capacity to induce Sanarelli-Shwartzman reaction is decreased by half as is the pro-coagulant activity measured with rabbit leukocytes [8,22,23].

The radiation treatment (10 Gy; ⁶⁰Co-gamma) of mice significantly increased their LPS sensitivity, and 300 μg doses led to 100% mortality on days 3 and 7 after treatment, whereas identical doses of RD-LPS induced no ill effects. It was also observed that animals bearing Lewis lung carcinomas showed increased sensitivity to LPS but RD-LPS had no ill effects in such animal [24].

It has long been recognized that bacterial endotoxins have significant metabolic effects. In the liver microsomal mono-oxygenase enzyme systems are sensitive to endotoxin [25]. The metabolic inactivation of narcotic agents depends on the microsomal enzyme activity. RD-LPS, in contrast with toxic LPS, exerts a significantly decreased inhibitory effect on liver microsomal mono-oxygenase system. In LPS-treated animals phenobarbital is unable to induce these enzymes,

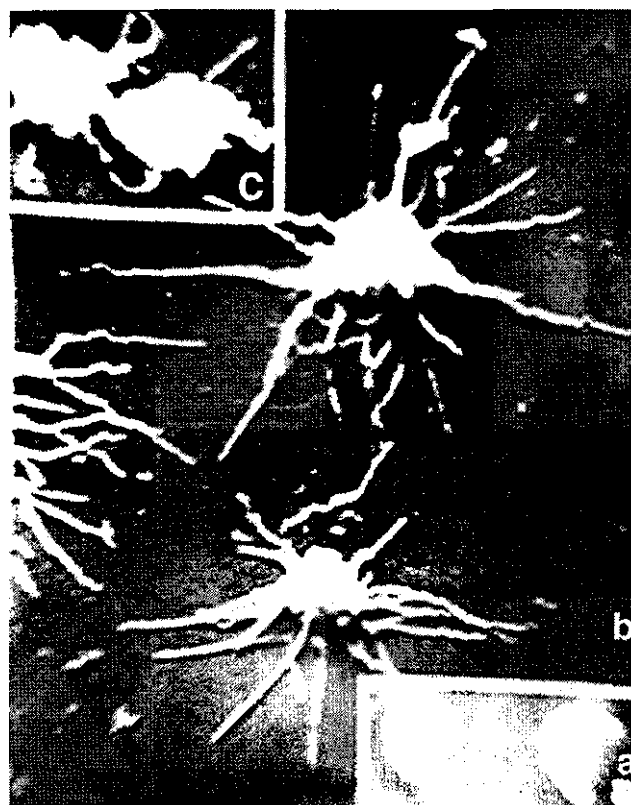


Fig. 1. Effect of endotoxin (LPS) and radio-detoxified endotoxin (RD-LPS: 150 kGy) on human thrombocytes (in vitro). Scanning electronmicroscopic studies (15,000x) (*n* = 5). (a) Untreated, (b) LPS-treated, (c) RD-LPS-treated (Kubasova, Somosy et al. cit. [52]).

whereas in RD-LPS (15 kGy) treated animals enzyme induction is present. The mechanism of the enzyme inhibition by LPS is unknown [25].

The dose-dependent induction of free radicals during irradiation in the water phase induces or leads to the induction of structural changes in endotoxin. It decreases the contents of glucosamine, keto-deoxy-octonic acid and fatty acids [26,27]. Ionizing radiation has been used in several other laboratories for the detoxification of endotoxin and confirmed our observations [17,28-32].

3. Interaction of RD-LPS with complement system

The complement system is fundamental to host defense, and the untreated toxic LPS activates this system. Ionizing radiation decreases in a dose-dependent manner the hypotensive (in dogs) and shock-inducing capacity (in dogs and rats) and complement-activating capacity of LPS. Thus, both the lethal and anti-complement effect of LPS was decreased by ionizing radiation. LPS is capable to activate complement by both the 'classical' and 'alternative' pathways. Although RD-LPS showed a dose-dependent decrease in the activation of both pathways, however, the activation of the alternative

pathway was less affected. This observation confirms our earlier findings that the two pathways are activated by different parts of the LPS molecule [33]. There is a close correlation between LPS toxicity and its complement-activating capacity. It was reasoned that the studies on complement activation may be used for the measurement of detoxification.

4. Effect of RD-LPS on endocrine system

LPS affects the membrane and TSH receptors of follicular cells in the thyroid gland [34,35]. In rats RD-LPS, although it decreases T4 (thyroxin) level in the serum, has no significant effect on the membrane and TSH receptors of follicular cells, and TSH is effective in the stimulation of T4 production. This indicates that RD-LPS is not capable of inducing membrane alterations, which damages membrane receptors, and yet it affects membranes as indicated by the induction of endotoxin tolerance [36,37]. Similar results have been found in irradiated animals and in various experimental shock models [35,38,39].

It is known from experiments in rats that LPS - either by direct effect or via cytokines - increases the circulating level of stress hormones (ACTH, corticosteroid and beta-endorphin), and it activates the pituitary-adrenal axis. Such activation is present in the absence of the paraventricular nucleus, which secretes releasing neuropeptides acting on the hypophysis [40,41]. In contrast, RD-LPS does not increase significantly ACTH, corticosteroid or beta-endorphin levels. These results indicate that ionizing radiation destroys the capacity of LPS to induce cytokines and to activate the pituitary-adrenal axis [37,42].

5. Effect of RD-LPS on arachidonic acid metabolism in macrophages

During endotoxin tolerance the mediator production of macrophages is altered, which is considered to be of significance for the induction of natural immunity/resistance. Therefore, it has been warranted to study in rats the effect of RD-LPS on macrophages in comparison with unaltered LPS. Our results indicate that RD-LPS is capable of inducing macrophage activation similar to toxic LPS, and LPS tolerance is also induced. Its capacity to induce arachidonic acid production is significantly longer than its capacity to inhibit endotoxin shock [43].

6. Protection of RES by RD-LPS against radiation and alcohol-induced injury

The reticuloendothelial system (RES) plays an important role in host defense by the removal of foreign material. For this reason we have examined the effect of RD-LPS on RES activity. Isotope removal (^{99m}Tc -labelled nanoalbumon mi-

crocolloid) from the blood is very sensitive method to detect granuloplectic activity and efficiency. The clearance curve that describes the RES activity is exponential, and it is well characterized by the granuloplectic index. The ^{99m}Tc -labelled nanoalbumon microcolloid is taken up by liver, spleen and bone marrow cells. The extent of RES damage can be measured by the colloid clearance and organ distribution. The irradiation and chronic alcohol consumption decrease the colloid clearance as also phagocytic activities in the liver, spleen and bone marrow. Large doses of LPS damage the RES system whereas identical doses of RD-LPS actually stimulate the phagocytosis. Moreover, the harmful effects of damaging agents on RES activity could be decreased or fully prevented by the treatment with RD-LPS. It is likely that the increase of RES activity plays a role in the stimulation of natural immunity/resistance [44,45].

7. Effect of RD-LPS on nitric oxide (NO) production

During the study of pro-inflammatory mediators the importance of nitric oxide has been recognized. NO plays an important role in endotoxin-induced circulatory and metabolic alterations. LPS stimulates the production the inductive NO synthase enzyme (iNOS) in various cell types. This mechanism apparently contributes to the LPS toxicity. Effects of LPS and RD-LPS on the NO system have been clarified. LPS-treated (10ng/ml) macrophages (J774 cell line) produce iNOS, which results in an increased NO production. If, however, the cells are pretreated 24 h earlier with RD-LPS (10ng/ml), no NO response is detected after a subsequent LPS treatment. These results indicate that RD-LPS induced tolerance towards NO induction by toxic LPS [46].

8. Increase of natural resistance by RD-LPS, protection against various forms of shock, adjuvant and antitumor effects and possible mechanisms of action

8.1. Endotoxin shock

It is well known that an adequate dose of endotoxin is capable of inducing severe shock [47,48]. This phenomenon is readily induced in sensitive animals simply by LPS injection into the bloodstream or into the peritoneal cavity. RD-LPS pretreatment of rats, mice, hamsters, guinea pigs, dogs, piglets, horses and monkeys is capable of inducing full endotoxin tolerance. Tolerance will develop within 24 h, and it will disappear between 1 and 4 weeks. The protective effect of RD-LPS is further supported by the measurement of circulatory parameters. LPS causes major alterations in blood pressure and cardiac output. Pretreatment with RD-LPS moderated, or fully protected against, the hemodynamic effects of toxic LPS [49].

8.2. Peritonitis caused by intestinal bacteria and septic shock

The prevention of peritonitis due to fecal bacteria and of sepsis are important practical problems. The pretreatment with RD-LPS protects 90% of the animals against lethal peritonitis or septic shock [9,47,50]. These results have been confirmed by other investigators [51]. Results suggest that RD-LPS might be used prior to abdominal surgery for the elevation of natural immunity/resistance [7,9,52].

8.3. Hemorrhagic shock

Fine [53] presumed several years ago that endotoxaemia plays a role in the pathophysiology of hemorrhagic shock, which is a significant problem in surgery. The RD-LPS pretreatment of dogs results in survival of the majority (70%) of the animals affected by hemorrhagic shock [47,49,54].

8.4. Intestinal ischemic shock

The occlusion of the superior mesenteric artery (arteria mesenterica superior) leads to a severe intestinal ischemia in man, which frequently results in death even when the occlusion is successfully removed by surgery. In animals the experimental intestinal ischemia induced by temporary closure of the anterior mesenteric artery is an excellent model for the examination of that injury. It has been demonstrated earlier that LPS absorbed from the gut during the reperfusion plays an important role in the pathogenesis of this condition [48,53,55-57]. RD-LPS pretreatment in experimental intestinal ischemia saves the majority of rats (70%) from lethal intestinal ischemic injury [9,48].

8.5. Pulmonary shock

Bacterial endotoxins play an important role in the pathogenesis of the pulmonary shock. In animal models LPS evokes a characteristic neutrophilic granulocyte infiltration in the lungs. This condition can only be moderately induced by RD-LPS, and pretreatment with RD-LPS (endotoxin tolerance) protects against the LPS-induced lung infiltration [58].

8.6. Tourniquet shock

Bacterial endotoxins may play a significant role in the pathogenesis of this condition. Pretreatment with RD-LPS prevented in 60% of the rats endotoxin shock induced by the ischemia of the hind leg [9,59,60].

8.7. Fetal death and abortion induced by LPS

Bacterial endotoxins play a role in the pathogenesis of these conditions during the urogenital infections [61]. It is not possible to protect against LPS-induced abortion by pharmaceutical means [62,63]. RD-LPS hardly exerts any toxic

effect on the fetus, yet it protects in 90% of the animals against LPS-induced fetal death and abortion [62].

8.8. Radiation-induced disease

Bacterial endotoxins play an important role in the intestinal syndrome of radiation disease [9,64-66]. It is also known that ionizing radiation damages the bone marrow which results in deficient hematopoiesis. For these reasons, it has been warranted to study the effect of RD-LPS on radiation-induced disease. Our experiments have revealed that RD-LPS protects some 70% of rats from a lethal radiation dose [9].

8.9. Immunosuppression

It is well known that sublethal radiation significantly decreases the natural resistance and adaptive immunity, increases the susceptibility to infection, may lead to the activation of dormant infection, and could further sepsis induced by facultative pathogenic organisms. Thus, sublethal radiation damages all forms of immune function and leads to general immunosuppression [4,67]. Early observations in radiation biology have revealed an atrophy of immune organs (e.g. spleen, thymus, lymph nodes) and hypertrophy of the adrenal gland. The lymphoid organs represent the anatomical basis of host defense and for this reason it is understandable that the radiation damage of these organs leads to a profound or complete immunosuppression. It is imperative that after radiation the regeneration of the immune system is accelerated as much as possible. Such situations occur daily during radiation treatment of cancer patients. RD-LPS, given 21 days after radiation damage (at this point no adaptive immune response is present), produces significant improvements in immune function and accelerates immune regeneration in rats [67]. The radioresistant T-helper cells also seem to be important in mediating the immunostimulatory effect of RD-LPS [19].

8.10. Immunosuppression due to antilymphocyte serum

It is well known that transplant patients die because of infectious disease rather than inadequate surgical procedures. In most cases sepsis or endotoxaemia is the cause of death. These patients receive strong immunosuppressive agents in order to prevent graft rejections. It is of common knowledge that immunosuppressive drugs decrease natural resistance to a large extent, and for this reason the patients become susceptible even to bacteria that have moderate pathogenicity. Antilymphocyte serum (ALS) is also used in these patients as an immunosuppressive agent. RD-LPS is capable of inducing endotoxin tolerance and enhanced natural resistance in antilymphocyte serum in rats immunized with sheep red blood cells that antilymphocyte serum treatment that led to complete immunosuppression i.e. this does not prevent the induction of endotoxin tolerance in response to RD-LPS. Therefore, endotoxin tolerance and the increase of natural resistance are independent of the suppression of adaptive im-

Table 2
Protection by radio-detoxified endotoxin (RD-LPS) against various forms of experimental shock, radiation disease and infections

Experimental intervention/species	Protective value (%)
Endotoxin shock (LPS, rat, pig)	100
Radiation disease (X-ray or $^{60}\text{Co-}\gamma$, rat)	70
Septic shock (fecal peritonitis, rat)	90
Tourniquet shock (limb ischemia, rat)	60
Intestinal ischemic shock (AMSO, rat)	74
Hemorrhagic shock (hypotension, dog)	70
Abortion (LPS, rat)	50
Pasteurella infection (X-ray + infection, rat)	100
Klebsiella infection (rat)	90
Aujeszky virus infection (rat)	70
Immunosuppression (ALS, rat)	100

munity by antilymphocyte serum. A possible reason for this is that endotoxin tolerance is primarily related to B-cell function. On the basis of these experiments it is possible that RD-LPS would protect transplant patients from septicaemia and endotoxaemia [9,68]. The results of these experiments are summarized in Table 2.

8.11. RD-LPS as an immunological adjuvant

Bacterial endotoxins are excellent immunological adjuvants [67,69]. However, the use of endotoxins in vaccine production is limited due to the undesirable side effects (fever and local inflammation). Nevertheless, the efficacy of some combination vaccines is improved by the small amount of LPS present. RD-LPS maintains its adjuvant potential [67,69,70]. The adjuvant effect of RD-LPS could also be demonstrated by thymidine incorporation in human white blood cells treated with phytohemagglutinin [19]. For this reason it is possible that RD-LPS would be useful also for the potentiation of viral antigens. There is a tendency for the use of inactivated viral vaccines in order to prevent the reversion of attenuated viruses to pathogenic strains. However, inactivated viruses are less immunogenic, which requires the use of adjuvants for the production of acceptable vaccines. Good adjuvants are in short supply. This has warranted the trial of RD-LPS for the potentiation of viral vaccines. We produced a vaccine against foot and mouth disease with RD-LPS, which was superior to the vaccine in use when tested in mice and pigs. We also succeeded in the production of vaccines against equine and human influenza viruses, which were significantly better than the ones in use at present [71-73].

It should be noted that RD-LPS adjuvant also increases natural-innate immunity/resistance in the vaccinated animals that lasts up to 1-3 weeks. Therefore, it may provide protection prior to the induction of adaptive immunity [52].

Newborn mice become more susceptible to the lymphocytic choriomeningitis virus if treated with LPS or RD-LPS. This is related to the immunoadjuvant effect of LPS/RD-LPS [74]. Ionizing radiation does not affect the stimulation of the lymphoid system by LPS [74-76]. Similar results were obtained in experiments with *Hemophilus influenzae* [77,78].

Newborn germ-free miniature piglets treated with a single dose of RD-LPS in contrast with the untreated germ-free animals that had an atrophic immune system, showed a fully developed lymphoid system in a short period (10-14 days), which was comparable histologically to the immune system of conventional animals of similar age (Mandel and Bert6k; cit. [52]).

It is likely that natural resistance, or rather its decrease, plays an important role in tumor progression. It is known that killed bacterial cultures [79] and bacterial endotoxins exert antitumor activity [11,80]. It is unfortunate that the use of LPS is limited in this situation by the harmful side effects it may have. One of the most important cytokines induced by LPS is the tumor necrosis factor (TNF), which has been tested for tumor therapy with great expectations. Unfortunately TNF is responsible in part for LPS toxicity. Thus, the toxic effect is related to the antitumor activity. This assumption is supported by the observation that it is possible to inhibit most of the harmful effects of LPS by anti-TNF monoclonal antibodies. One should note, however, that a chemically detoxified toxin preparation, monophosphoryl lipid A, can exert some antitumor effect [11].

It appears to be very important that the LPS preparations can enhance the natural resistance [2,3,5]. It is of common knowledge that radiation treatment, chemotherapeutic agents, radiation sensitizing agents, steroids, local heat treatment and even surgical procedures induce a profound decrease in natural resistance. For this reason the patients become sensitive to infectious agents and a significant proportion fall victims to sepsis caused by Gram-negative bacteria, become endotoxaemic or develop pneumonia. Anticancer agents as well as ionizing radiation are harmful not only to tumor cells but also to other cells, especially to the membranes. For instance, formyl-leucosine (Kobanyai Gyogyszeruigy, Budapest), which was effective in animal experiments, could not be used on patients because of cardiotoxicity. It causes hypotonia and membrane damage of cardiac muscle [81]. For this reason we tested the effect of RD-LPS pretreatment on formyl-leucosine cardiotoxicity. It has been observed in dogs that RD-LPS pretreatment has a minimal effect on the hypotensive effect of formyl leucosine; however, after 30min blood pressure and cardiac function return to normal. Examination of the cardiac muscle of RD-LPS treated dogs did not show the pathological changes of membrane injury, which was present in formyl-leucosine treated animals [82]. This experiment indicates that treatment with RD-LPS may protect against the side effects of chemotherapeutic agents and radiation treatment.

It is possible that the mechanism of protection by RD-LPS is mediated by the so-called lysosomal membrane 'concentration'. In RD-LPS tolerant animals no lysosomal membrane damage occurs after LPS challenge and consequently lysosomal enzymes are not released (beta-glucuronidase, cathepsin D, etc.) [8].

Most of the desirable effects of LPS are preserved in RD-LPS. These are RES activation, stimulation of phagocytosis

and macrophage activity, elevation of natural antibody and properdin levels. RD-LPS activates the entire immune system, which includes both humoral and cellular immunity. This is the reason for the effectiveness of RD-LPS against diverse noxious agents [9]. It is possible that RD-LPS will gain application in complex therapeutic approaches to neoplasia.

8.12. Hepatoprotective effect of RD-LPS in experimental myocardial infarction

We have examined in dogs whether or not RD-LPS would protect against liver damage caused by myocardial infarction elicited by the occlusion of the left coronary artery. It was observed that in RD-LPS treated dogs there was a moderate or no disturbance of permeability, and liver function including detoxification remained normal [47,83]. These results indicate that RD-LPS may be useful in several clinical situations.

8.13. Effect of RD-LPS on hematopoiesis

Endotoxin affects bone marrow function [84]. Numerous drugs (such as chemotherapeutic agents) and medical procedures (radiation therapy) impair the host defense system. Damage to the bone marrow and lymphatic system leads to the decrease of natural-innate immunity/resistance. The number of stem cells will decrease in the bone marrow, which leads to a decreased production of white blood cells (especially granulocytes) and agranulocytosis will develop.

The RD-LPS treatment of rabbits can improve or restore bone marrow function, when the animals are given immunosuppressive agents (Immuran, hydrocortisone) (Table 3). Comparative blood counts indicate that RD-LPS increases significantly the production of white blood cells in healthy animals, and it restores almost completely the bone marrow function in Immuran- and hydrocortisone-treated rabbits (Gaboretal.; cit. [52]).

8.14. Effect of RD-LPS on apoptosis and stimulation of HIV-infected lymphocytes

HIV-infected lymphocytes show increased apoptosis. Lymphocytes isolated from healthy but HIV-infected individuals ($CD4 < 500 \text{ jxl}^{-1}$) and treated in vitro with interleukin-2 (hrIL-2) show increased apoptosis if treated with HIV peptides. RD-LPS inhibits this reaction in over 60 percent of the experiments. These experiments suggest that the use of RD-LPS may be helpful in the prevention of apoptosis in HIV-infected individuals (Nagy et al.; cit. [52]).

There is an asymptomatic phase after HIV injection if the virus proliferates in the lymph nodes. The deficiency of $CD4+$ T-lymphocytes is central to the development of acquired immunodeficiency syndrome (AIDS). It is possible that the stimulation of cell cycle, by the restoration of immune function, may delay the development of AIDS. Studies on lymphocytes from patients with different stages of HIV infection for the reaction to RD-LPS and to some other agents

Table 3
Protection by radio-detoxified endotoxin (RD-LPS) against leukopenia in Immuran or hydrocortisone pretreated, immunosuppressed rabbits

Group	Basal volume			Before RD-LPS treatment after immunosuppression			After 2 weeks of first RD-LPS treatment			After 4 weeks of first RD-LPS treatment			After 4 weeks second RD-LPS treatment		
	I (10^3)	2 (%)	3 (%)	I (10^3)	2 (%)	3 (%)	I (10^3)	2 (%)	3 (%)	I (10^3)	2 (%)	3 (%)	I (10^3)	2 (%)	3 (%)
(1) Control	7.9	73	27	7.4	70	30	7.9	63	37	8.5	70	30	8.8	63	37
(2) RD-LPS	8.6	58	42	8.6	76	24	16.7	84	16	16.8	89	11	14.5	90	10
(3) Immuran + RD-LPS	7.0	76	24	5.5	82	10	7.9	77	23	8.6	82	18	6.9	82	18
(4) Hydrocortisone + RD-LPS	8.0	64	36	4.1	70	30	6.1	68	32	5.4	81	19	-	-	-

(1) Leukocytes, (2) lymphocytes, (3) granulocytes.

indicate that during the initial stage of AIDS RD-LPS stimulates immature T-lymphocytes and delays via immune the activation of the AIDS syndrome development [85].

9. Evaluation of RD-LPS for human use on volunteers

Experiments described below have been performed with the permission of the Hungarian Ministry of Health using human volunteers. The first experiment included five subjects (four male and one female). It was observed that RD-LPS given at the dosage level of 7 fxg/kg (1 mg/ml solution was used) subcutaneously caused no adverse effects (circulatory, respiratory, urinary tract, cardiac function, nausea, vomiting or diarrhea) within the 24-h observation period. A moderate febrile reaction, an increase in blood pressure and some swelling at the injection site were observed. These symptoms significantly diminished or disappeared completely within the next further 24-h observation period. The repeated laboratory testing revealed no changes in blood coagulation, liver and kidney function. In contrast, the C3 complement component was elevated in the blood and at 24 h so was the leukocyte count. On this basis it may be concluded that the dose applied does not elicit a greater reaction than vaccines do, and may be considered harmless and suitable for further clinical studies.

For the second experiment 40 volunteers were recruited from university students. We observed that RD-LPS administered subcutaneously at a dose of 4 fxg/kg did not produce any clinical symptoms (circulatory, respiratory, urinary, cardiac damage, nausea or diarrhea) at all. At various times after application there was a moderate elevation in body temperature with a maximum of 37.9 °C in one individual. There was a moderate swelling at the site of application. These changes disappeared within 24 h after the application. The laboratory tests performed at 24 h did not show any alteration in blood coagulation, liver and kidney function. In the serum of treated individuals, taken at 24 h, tumor necrosis factor was detected. There was also a moderate elevation of serum T4 (thyroxin) in most individuals. The leukocyte count and the C3 complement fraction in the serum were also increased. The treated individuals were followed for 3 weeks, and two individuals developed a mild form of cold syndrome during that period. It may be concluded on the basis of both experiments that TOLERIN is essentially harmless and it causes only a very mild clinical reaction and therefore it is suitable for further clinical trials.

9.1. Clinical trials with RD-LPS

After our initial clinical trials had been completed, the Hungarian National Agency of Public Health and Infectious Diseases gave permission for the trial of RD-LPS registered as TOLERIN® in nine health institutions in both open and double-blind experiments. During these studies 110 surgi-

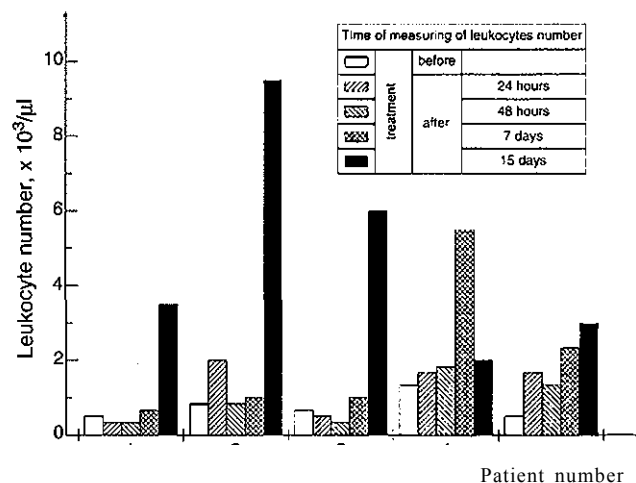


Fig. 2. Effect of radio-detoxified endotoxin (RD-LPS: TOLERIN) on the number of leukocytes in CYSPLATIN treated patients bearing testis tumors.

cal patients were treated with it, and 241 patients receiving similar medical treatment served as control. The majority of patients had been operated for colon carcinoma. TOLERIN was given to patients 24-48 h prior to surgery. It has been observed so far that in the treated individuals there was a decrease of infections and complications and natural resistance was increased on the basis of laboratory tests (increase in white blood cell count and C3 complement levels).

We also studied testicular carcinoma patients treated with Cysplatin for the correction of granulocytopenia. The experiment performed on five patients is presented in Fig. 2 (Bodrogi et al.; cit. [52]). TOLERIN treatment stimulated bone marrow function, and leukocyte and granulocyte counts were increased significantly. This experiment indicates that such treatment may be used for the correction of bone marrow suppression caused by chemotherapeutic agents.

We also treated five individuals suffering from immunodeficiency in addition to the usual treatment regimes given to these patients. Although TOLERIN increased CD4 number in patients with full-blown AIDS, no clinical improvement was obvious in the treated individuals. This observation correlates with our in vitro studies performed earlier. However, in one patient aged 28 years there was a significant clinical improvement after the treatment. This patient suffered from severe meningitis and encephalitis of unknown origin, *Candida* sepsis and of mixed bacterial infections. The CD4+ and CD8+ T-cell count was 250 and 261, respectively. TOLERIN was given two times at 280 μg subcutaneously (the second treatment was given 60 days after the first), and it increased significantly the CD4+ and CD8+ lymphocyte count. There was improvement already after the first treatment and after the second treatment the CD4+/CD8+ ratio was 1.05. One year later the CD4+/CD8+ ratio increased to 2.0, which is slightly over normal. Bone marrow function was normalized by this treatment [86].

10. Conclusions

Both the literature summary on the action mechanisms and pathophysiology of LPS and RD-LPS as well as the first clinical trials suggest that RD-LPS may have a wide usability in medicine. Further experimental and clinical studies are warranted.

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