Natural Immunity
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New Prospect for the Enhancement of Natural Immunity.

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ABSTRACT

It is well demonstrated that serial endotoxin injections produce endotoxin tolerance and elevate natural resistance. However, such injections may also*have-harmful effects such as high fever, hypotension and abortion. For this reason LPS^injections are not suitable to enhance nonspecific resistance in endotoxin-sensitive mammalian species including man. Various techniques have been designed (physical, chemical, etc.) for the detoxification of endotoxins while the beneficial effects were maintained. Perhaps one of the best detoxification techniques is treatment with ionizing radiation. The irradiation of LPS with ^{60}Co (100-200 kGy) decreased its toxicity in a dose-dependent manner. Such radiodetoxified endotoxin (RD-LPS) preparations showed decreased toxicity whereas the beneficial effects were preserved (150 kGy:TOLERIN®). These findings have been confirmed in other laboratories. Irradiation causes marked chemical alteration in LPS, such as the decrease of glucosamine, KDO and fatty acids. A single parenteral injection of TOLERIN® is capable of preventing the various forms of shock in experimental animals. This preparation has a membrane-stabilizing effect and thereby can prevent the membrane-damaging effect of LPS and of some cytostatic agents. Unlike endotoxin, TOLERIN has barely any hypotensive effect and pretreatment with this preparation can prevent practically all the haemodynamic changes induced by LPS. LPS plays an important role in the pathogenesis of the intestinal syndrome of radiation disease, which may be prevented by up to 70% in rats with RD-LPS pretreatment. TOLERIN retains the adjuvant activity of LPS and it is a good adjuvant for inactivated virus vaccines. TOLERIN 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, endotoxaemia, pneumonia and so on. 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. We found that in ALS-treated rats RD-LPS induced tolerance against the lethal dose of LPS. This experiment demonstrated that in spite of the suppressive effect of ALS on T lymphocytes the induction of LPS tolerance (the enhancement of natural resistance) was normal. Facultative pathogenic organisms may flourish and cause disease when specific and nonspecific resistance is impaired. RD-LPS could produce 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, antitumour activity, etc. On the basis of these favourable experimental results, TOLERIN was tested on 350 surgical patients suffering from gastrointestinal tumours, on other patients suffering from AIDS and on cancer patients treated with CYSPLATIN©. TOLERIN treatment prevented sepsis and activated bone marrow function in these patients.

1. INTRODUCTION

At the beginning of the last century Wright [1] first studied natural resistance (NR). He observed that after vaccination with killed bacteria a 'negative' phase occurred which was followed by increased natural resistance tied to the production of specific antibodies. Landy [2] and Rowley [3] made similar observations later independently, and 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 originally discovered by Pillemer et al. [5], was also stimulated by LPS. Beeson [6] discovered that low doses of toxic LPS given repeatedly led to the induction of endotoxin tolerance and prevented 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 tumour necrosis: it stimulated bone marrow activity and increased the production of interferon. Moreover, it was possible to prevent the development of infectious diseases by small endotoxin doses, which increased natural resistance significantly. These observations evoked great interest in the phenomenon of endotoxin tolerance [7].

The question was posed whether or not endotoxin tolerance could be used for the prevention of endotoxin shock and of shock due to other causes. It was clear that during endotoxin tolerance no lysosomal membrane damage ocpurred even if LPS was given repeatedly [8], and the animals survived the endotoxin challenge [9]. However, the use of endotoxin for preventive treatment was limited by the excessive sensitivity of man and of higher animals to LPS. For this reason, numerous attempts were made using physical, chemical and immunological approaches to produce LPS preparations that would maintain its ability to induce tolerance, increase natural resistance, act as an adjuvant and exert a necrotic effect on tumours while decreasing or entirely eliminating its toxicity. Methylation [10] and the modification of the structure of LPS molecule of the so-called monophosphoryl lipid A [11-13] were successful in detoxifying LPS but the capacity to induce natural resistance was best preserved when ionizing radiation (^{60}Co-gamma) was used for detoxification [14-17].

2. THE PRODUCTION AND BIOLOGICAL EFFECTS OF RADIODETOXIFIED LPS

In our laboratory E. coli bacteria are produced in bulk by fermentation and extracted by the phenol-water method of Westphal with some modifications [14]. The LPS is further purified by ultracentrifugation and dissolved in water by $^{60}Co_{\gamma}$ gamma radiation using 50, 100, 150 or 200 kGy doses. The toxicity of LPS is decreased as shown in Fig. 1.

The biological effects of radiation-treated LPS are presented in Table I. The harmful effects of LPS are decreased after radiation, whereas its capacity to induce tolerance, to function as an immunoadjuvant and immunomodulator, to protect against shock and radiation and to stimulate natural resistance are preserved to a large extent. The lethal effect of such preparations (LD_{50}

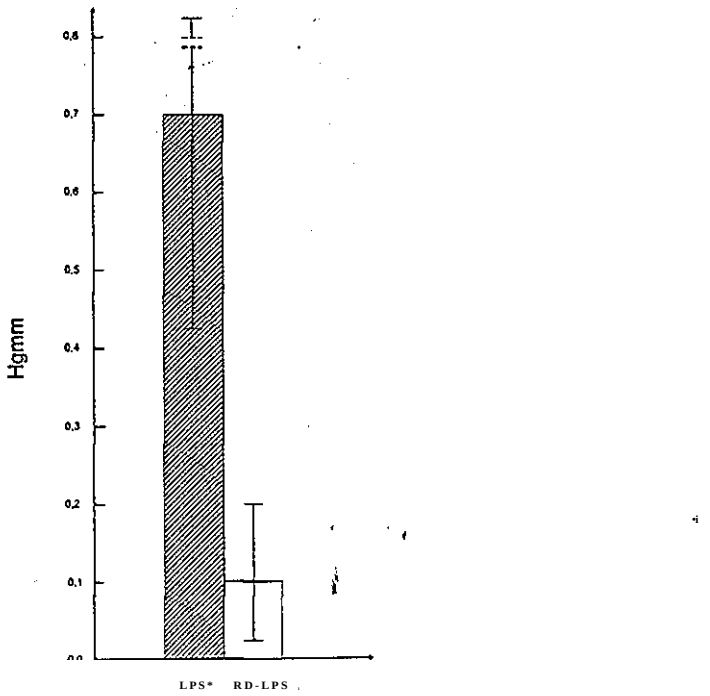

Figure 1. Blood pressure decreasing effect of endotoxin ({.PS) and radiodetoxified endotoxin (RD-LP$: 200 kGy) in dogs 4-6 minutes after the treatment, (mean ±SEM for LPS:61±24 Hgmm; RD-LPS: 9.8±8.S Hgmm; with n=14; P<0.01).

Table I Comparison of various biological effects of intact endotoxin (LPS) and radiodetoxified (5 Mrads) E.coli endotoxin (radiodetoxified endotoxin) preparations.

Effects	LPS	RD-LPS
LD50 [rat, mg/kg, n=50]	20	50
Hypotension [dog, Hgmm, n=10]	<60	>10
Leukocyte decrease [rabbit, % , n=10]	66	45
Platelet decrease [rabbit, %]	80	50
Fibrinogen decrease [rabbit, %. n=10]	40	17
Reptilase time increase [rabbit, /%]	90	10
Pyrogenecity [rabbit, ° C, n=10]	2.4	1.2
Sanarelli-Shwartzman local [rabbit, mm^2 , n=5]	1200	600
Hyperlipidemic effect [mouse, n=20]	+	–
Fetopathy [rat, % , n=50]	100	2
Complement depletion in vivo [rabbit, %]	35	10
Spermatozoon agglutination in vitro [swine, n=50]	+	–
Anticomplement activity in vitro [μg]	30	174
Adjuvant effect [rat, %, n=100]	508	470
Interferon induction [mouse, n=40]	+	+
Stem cell mobilization [CFUS /mouse, n=25]	521	247
Endotoxin tolerance [rat, pig, n=200, 10, protection, %]	100	100
Shock preventing [rat, dog, n=250; 10, protection, %]	80	80
Radioprotective effect [rat, n=300, protection, %]	70	70

and LDM) is dependent on the amount of radiation applied [14,16,17]. It was shown that the anti-complement and platelet-aggregating effect (indicating membrane damage) of detoxified endotoxin was partly maintained [18], which was dependent on the radiation dose used (Fig. 2). The decrease of membrane perturbation by radiated LPS was proven in these studies by the measurement of ^{3}H-concanavalin binding [19]. Table I shows the comparison of major effects

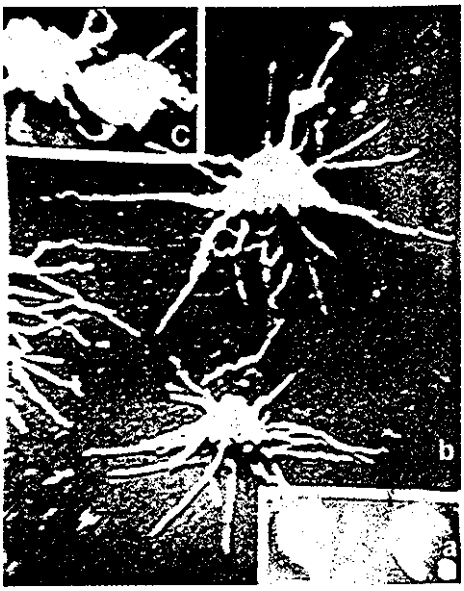

Figure 2. Effect of endotoxin (LPS) and radiodetoxified endotoxin (RD-LPS: 150 kGy) on human thrombocytes (in vitro). Scanning electronmicroscopic studies (15000X) (n=5). (a) untreated, (b) LPS-treated, (c) RD-LPS-treated [18].

of LPS and RD-LPS.

It was demonstrated in mice that toxic LPS increased the blood level of very low density lipoprotein (VLDL) whereas the RD-LPS did not, indicating that hyperlipidaemia is a marker of LPS toxicity [20]. We also found that RD-LPS inhibited membrane-bound adenylate cyclase (AC) less 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, cathepsin D). The capacity to induce the Sanarelli-Shwartzman reaction was decreased by half, as was pro-coagulant activity as measured with rabbit leukocytes [8,22,23].

We found that radiation treatment of mice (10 Gy; ^Co-gamma) significantly increased their LPS sensitivity and 300 |xg 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 animals [24].

It is long recognized that bacterial endotoxins have significant metabolic effects. In the liver microsomal monooxygenase enzyme systems are sensitive to endotoxin [25]. Because of the potential anti-shock effect of RD-LPS, we examined its effect on the microsomal monooxygenase system. The metabolic inactivation of narcotic agents is dependent on microsomal enzyme activity. We demonstrated that RD-LPS, in contrast with toxic LPS, exerts a significantly decreased inhibitory effect on liver microsomal mono-oxygenase enzymes. In LPS-treated animals phenobarbital was unable to induce these enzymes, whereas in RD-LPS (150 kGy) treated animals enzyme induction was present. The mechanism of enzyme inhibition by LPS is unknown. It is-possible that LPS damages the cytochrome P-450 enzyme [25].

We studied the mechanism of endotoxin detoxification by radiation. It was indicated that a dose-dependent induction of free radicals in the water phase induced or led to the induction of structural changes in endotoxin. This led to the decrease 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].

4. THE EFFECT OF RD-LPS ON THE ENDOCRINE SYSTEM v

4.1. Thyroid gland $ - .

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

4.2. Pituitary-adrenal axis

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, beta-endorphin) and activates the pituitary-adrenal axis. Such activation is present in the absence of the paraventricular nucleus, which secretes releasing neuropeptides towards 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].
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5. THE EFFECT OF RD-LPS ON ARACHIDONIC ACID METABOLISM IN MACROPHAGES.

Endotoxin tolerance is so far the best model to study natural resistance. For this reason it is imperative that we understand its mechanisms. During the mediator production of macrophages is altered, which is considered to be of significance for the induction of natural resistance. Therefore, it was 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

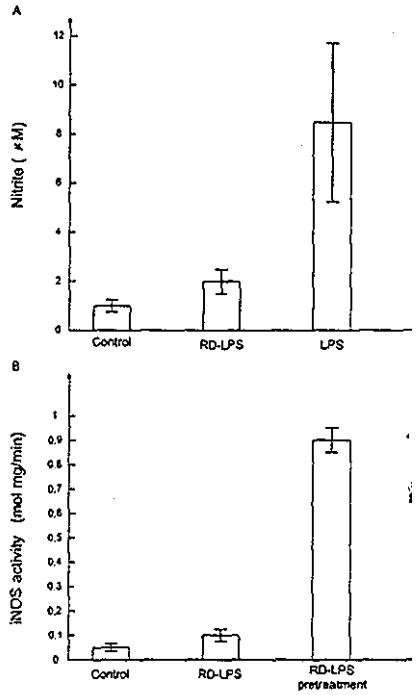

Figure 3. Nitrite production (A) and iNOS activity (B) by J774 macrophages exposed to DMEM **only (control),** RD-**LPS or native** LPS **(10 ng/ml) for 24** h. **Each value represents the mean ±** SEM **of N=18 wells from three independent experiments.** Pc **0.01 versus control.**

A. NO formation	**control:**	**I ± 0.8 uM**	**B. iNOS activity**	**control:**	**0.1 ± 0 pM(mg/min)**[-1]
	RD-LPS:	**2.3 ± 2.0** uM		RD-LPS:	**0.3 ± 0.01** pMOng**/min)**[-1]
	LPS:	**8.3 ± 3.8** uM		LPS:	**0.9 ± 0.02** pMOng**/min)**[-1]

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similar to toxic LPS, and LPS tolerance is>also induced. Its capacity to induce arachidonic acid production persisted significantly longer than its capacity to inhibit endotoxin shock [43].

6. THE PROTECTION OF RES BY RD-LPS AGAINST RADIATION AND ALCOHOL-INDUCED INJURY

The reticuloendothelial system (RES) plays an important role in host defence by the removal of foreign material. For this reason we have examined the effect of RD-LPS on RES activity. A method of isotope (^{99m}Tc-labelled nanoalbumon microcolloid) removal from the blood was established. This method is very sensitive and is suitable for the detection of grahulopetic activity and efficiency. The clearance curve that described RES activity was of exponential nature and it was well characterized by the granulopectic index. The liver, spleen and bone marrow cells took up the ""Tc-labelled nanoalbumon microcolloid. The extent of RES damage was measured by colloid clearance and organ distribution. The effect of radiation and chronic alcohol consumption has.been investigated. Under these conditions colloid clearance was decreased, as was phagocytic activity in the liver, spleen and bone marrow. It was shown that large doses of LPS damaged the RES system, whereas identical doses of RD-LPS were actually stimulatory on phagocytosis. Moreover, the harmful effects of damaging agents on RES activity could be decreased or fully prevented by treatment with RD-LPS. It is likely that the increase in RES activity plays a role in the stimulation of natural resistance [44,45].

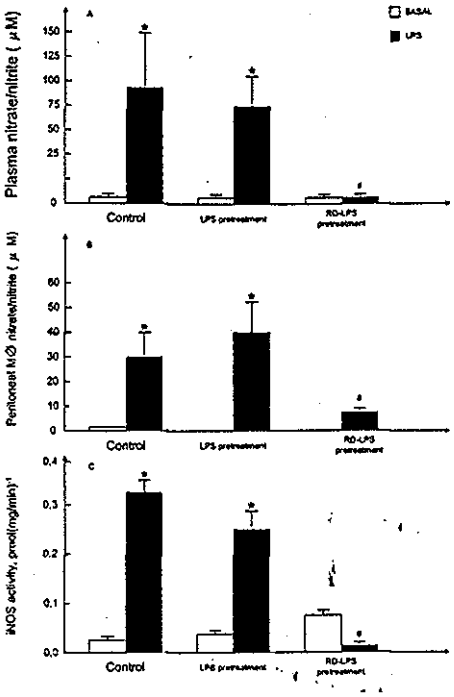

Figure 4. Effect of RD-LPS or LPS pretreatment in rats on rjlasma nitrate/nitrite levels (A), peritoneal macrophage (Mr) nitrate/nitrite levels (B) and lung iNOS activity (C) after subsequent endotoxin challenge. Rats, given sublethal doses of RD-LPS, LPS (1 mg/kg i.p.) or saline solution only (control) for four days, were challenged with a high dose of LPS (15 mg/kg i.p.) on the fifth day. Open columns represent basal values before lethal LPS challenge; filled columns represent values obtained 6 h after high-dose LPS challenge. Each value represents the mean ± SEM of five rats for each group. *P < 0.01 versus control. # P < 0.01 versus LPS pretreatment.

7. THE EFFECT OF RD-LPS ON NITRIC OXIDE (NO) PRODUCTION

During the study of pro-inflammatory mediators the importance of nitric oxide (NO) has been recognised. NO plays an important role in endotoxin-induced circulatory and metabolic alterations. LPS stimulates the production of the inductive NO synthase enzyme (iNOS) in various cell types. This mechanism contributes to LPS toxicity. For this reason we have examined the effect of LPS and RD-LPS on the NO system. LPS-treated (10 ng/ml) macrophages (J774 cell line) produced iNOS, which resulted in increased NO production. If, however, the cells were pretreated 24 hours earlier with RD-LPS (10 ng/ml), no NO response was detected after the subsequent LPS treatment. In contrast, when the cells were pretreated with LPS, no change was observed in the NO response to subsequent LPS challenge. The treatment of rats with repeated LPS injections (1 mg/kg/day ip. for 4 days) prevented the rise of nitrate/nitrite levels in the blood. These results indicate that RD-LPS induced tolerance towards NO induction by toxic LPS [46] (Figs. 3 and 4).

8. RD-LPS INCREASES NATURAL RESISTANCE, PROTECTS AGAINST VARIOUS FORMS OF SHOCK, AND EXERTS ADJUVANT AND ANTITUMOUR EFFECTS. 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 blood-stream or into the peritoneal cavity. We demonstrated that RD-LPS pretreatment of rats, mice, hamsters, guinea pigs, dogs, piglets, horses and monkeys was capable of inducing full endotoxin tolerance. Tolerance will develop within 24 hours and will disappear between 1 and 4">weeks. The protective effect of RD-LPS was further supported by the measurement of circulatory parameters. LPS caused major alterations in blood pressure and cardiac output. Pretreatment with RD-LPS moderated, or fully protected against, the haemodynamic effects of toxic LPS [49].

8.2. Peritonitis caused by intestinal bacteria and septic shock

The prevention of peritonitis due to faecal bacteria and of sepsis is important in clinical practice. Therefore, we studied the effect of RD-LPS in experimental models of these conditions. It was shown that pre-treatment with RD-LPS protected 90% of the animals against lethal peritonitis or septic shock [9,47,50]. Other investigators confirmed these results [51]. It is indicated that RD-LPS may be used prior to abdominal surgery for the elevation of natural resistance [7,9,52].

8.3. Haemorrhagic shock

Haemorrhagic shock is a significant problem in surgery. Fine and co-workers [53] presumed several years ago that endotoxaemia plays a role in the pathology of this condition. For this reason we studied the effect of RD-LPS on haemorrhagic shock and demonstrated that the pre-treatment of dogs resulted in survival of the majority (70%) of the animals affected by lethal shock [47,49,54].

8.4. Intestinal ischaemic shock

The occlusion of the superior mesenteric artery (artéria mesenterica superior or cranialis) leads to severe intestinal ischaemia in man, which frequently results in death even when the occlusion is successfully removed by surgery. In animals the experimental intestinal ischaemia induced by temporary closure of the superior mesenteric artery is an excellent model for the examination of that injury. It has been demonstrated earlier that LPS absorbed from the gut during reperfusion plays an important role in the pathogenesis of this condition [48,53,55-57]. We studied the effect of RD-LPS in experimental intestinal ischaemia and showed that the majority of rats (70%) were saved from lethal intestinal ischaemic injury by our preparation [9,48].

8.5. Pulmonary shock

This condition is of clinical importance and bacterial endotoxins play an important role in its pathogenesis. In animal model systems LPS is used for induction, which evokes a characteris-

Table II **Protection by radiodetoxified 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 60Co-γ, rat]	70
Septic shock [fecal peritonitis, rat]	90
Tourniquet shock [limb ischemia, rat]	60
Intestinal ischemic shock [AMSO, rat]	74
Haemorrhagic 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

tic neutrophilic granulocyte infiltration into the lungs. TJiis condition can only be moderately induced by RD-LPS, and pretreatment with radiodetoxified 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 as well. Pre-treatment with RD-LPS prevented endotoxin shock induced by ischaemia of the hind leg in 60% of the rats tested [9,59,60].

8.7. Foetal death and abortion induced by LPS

Bacterial endotoxins play a role in the pathogenesis of these conditions during urogenital infections [61]. It is not possible to protect against LPS-induced abortion by pharmaceutical means [62,63]. Therefore, we studied whether or not RD-LPS would prevent LPS-induced foetal death and abortion. We found that RD-LPS hardly exerts any toxic effect on the foetus, yet it protects 90% of the animals against LPS-induced foetal death and abortion [62].

8.8. Radiation-induced disease

It has been known for some time that the toxic form of LPS in small doses protects against radiation, but such treatment was not practical because of the imminent danger of toxicity. We showed earlier that 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 haematopoiesis. For these reasons, it was warranted to study the effect of RD-LPS on radiation-induced disease. Our experiments revealed that RD-LPS protected 70% of rats from a lethal radiation dose [9]. This result prooves the effectiveness of this preparation.

8.9. Immunosuppression

It is well known that sub-lethal radiation significantly decreases natural resistance and adaptive immunity, increases the susceptibility to infection, may lead to the activation of dormant infection, and could lead to further sepsis induced by facultative pathogenic organisms. Thus,

sub-lethal radiation damages all forms of immune function and leads to general immunosuppression [4,67]. Early observations in radiation biology revealed the atrophy of immune organs (e.g., spleen, thymus, lymph nodes) and the hypertrophy of the adrenal gland. The lymphoid organs represent the anatomical basis of host defence and for this reason it is understandable that radiation damage to these organs leads to a profound or complete immunosuppression. It is imperative that after radiation the regeneration of the immune system be accelerated as much as possible. Such situations occur daily during radiation treatment of cancer patients. We have examined the effect of RD-LPS on immune regeneration after non-lethal irradiation in rats. RD-LPS given 21 days after radiation damage (at this point no adaptive immune response was present) produced significant improvements in immune function and accelerated immune regeneration [67]. Radioresistant T-helper cells seem to be important in the mediation of the immunostimulatory effect of RD-LPS [19].

8.10. Immunosuppression due to antilymphocyte serum

RD-LPS induces endotoxin tolerance and elevates natural resistance. 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 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 is also used in these patients as an immunosuppressive agent. We examined whether or not RD-LPS is capable of inducing endotoxin tolerance and enhanced natural resistance in antilymphocyte serum treated animals. It was found in rats immunized with sheep red blood cells that antilymphocyte serum treatment, that led to?complete immunosuppression, did not prevent the induction of endotoxin tolerance in response to RD-LPS. Therefore, endotoxin tolerance and indeed tha increase of natural resistance was independent of the suppression of adaptive immunity 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 II.

8.11. RD-LPS as an immunological adjuvant

It has long been known that bacterial endotoxins are excellent immunological adjuvants [67,69]. However, the use of endotoxins in vaccine production was limited due to the undesirable side effects (fever, local inflammation). Nevertheless, the efficacy of some combination vaccines is improved by the small amount of LPS present. We observed in rats that 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 phytohaemagglutinin [19]. For this reason it is possible that RD-LPS would be useful also for the potentiation of viral antigens. Inactivated viral vaccines are often used in order to avoid the danger of 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 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 (these findings are the subject of several patents) [71-73].

It should be noted that the RD-LPS adjuvant also increased natural resistance in the vaccinated animals that lasted for 1 to 3 weeks. Therefore, it provides natural 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 Haemophilus influenzae [77,78].

In other experiments we treated newborn germ-free miniature piglets aged a few days with a single dose of RD-LPS and examined the effect on the lymphoid system. In contrast with untreated germ-free animals that had an underdeveloped immune system, the animals treated with RD-LPS 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 [52].

It is likely that natural resistance, or rather its decrease, plays an important role in tumour progression. It is known that killed bacterial cultures [79] and bacterial endotoxins exert antitumour activity [11,80]. It is unfortunate that the use of LPS is limited again in this situation by the harmful effects it may have. One of the most important cytokines induced by LPS is tumour necrosis factor (TNF), which has been tested for tumour therapy with great expectations. Unfortunately, the expected results did not materialize. It became clear that TNF is responsible in part for LPS toxicity. Thus, the toxic effect is related to the antitumour 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, exerts some anti-tumour effect [11].

It is very important that LPS preparations enhance natural resistance [2,3,5]. It is 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 victim to sepsis caused by Gram-negative bacteria, become endotoxaemic or develop pneumonia. Anticancer agents as well as ionizing radiation are harmful not only to tumour cells but also to normal cells, especially to the membranes. For instance, formyl-leurosine (Kőbányai Gyógyszerárugyár, 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-leurosine cardiotoxicity. It was observed in dogs that RD-LPS pretreatment had a minimal effect on the hypotensive effect of formyl leurosin; however, after 30 minutes blood pressure and cardiac function returned to normal. Examination of the cardiac muscle of RD-LPS treated dogs did not show the pathological changes of membrane injury, which were present in formyl-leurosine 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 with RD-LPS treatment. These are RES activation, stimulation of phagocytosis and macrophage activity, elevation of natural antibody

and properdin levels, and so on. RD-LPS is capable of activating 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. Hepato-protection by RD-LPS in experimental myocardial infarction.

We 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 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. The effect of RD-LPS on haematopoiesis

It has long been known that endotoxin affects bone marrow function [84]. Numerous drugs (such as chemotherapeutic agents) and medical procedures (radiation therapy) impair the host defence system. Damage to the bone marrow and lymphatic system leads to the decrease of natural 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.

For these reasons we examined whether or not RD-LPS treatment could improve or restore bone marrow function under these conditions. Rabbits were used for these experiments. The animals were given immunosuppressive agents (Imuran, hydrocortisone) in order to suppress and damage the bone marrow. Comparative bldod count was performed after RD-LPS treatment. RD-LPS increased significantly the production of white blood cells in healthy animals and restored, alrnost completely bone marrow function in Imuran- and hydrocortisone-treated rabbits [52].

8.14. The effect of RD-LPS on apoptosis

It is known that HIV-infected lymphocytes show increased apoptosis. We studied the effect of RD-LPS on this phenomenon. Lymphocytes were isolated from healthy HIV-infected individuals (CD4 < 500/ul) and treated in vitro with interleukin-2 (hrIL-2). Twenty-four hours later the cells were treated with HIV peptides or with RD-LPS. It was observed that the HIV peptides increased apoptosis in all experiments, whereas RD-LPS inhibited this reaction in over 60 percent of the experiments. These experiments suggest the use of RD-LPS for the prevention of apoptosis in HIV-infected individuals [52].

8.15. The effect of RD-LPS on the stimulation of HIV-infected lymphocytes

In man there is an asymptomatic phase after HIV injection, while the virus proliferates in infected lymphocytes and macrophages. The loss of CD4+ T lymphocytes is central to the development of acquired iramunodeficiency,syndrome (AIDS). It is possible that the augmentation of immune function would delay the development of AIDS. Therefore, we examined lymphocytes from patients with different stages of HIV infection for the reaction to RD-LPS and to some other agents. These studies indicated that during the initial stage of AIDS RD-LPS stimulated

immature T-lymphocytes [85].

9. THE EVALUATION OF RD-LPS (TOLERIN®) FOR HUMAN USE ON VOLUNTEERS

These experiments have been performed with the permission of the Hungarian Ministry of Health, using human volunteers. The first experiment included five subjects (4 male and 1 female). It was observed that TOLERIN given at the dose of 7 ug/kg subcutaneously (1000 u.g/ml solution was used) caused no adverse effects (circulatory, respiratory, urinary tract, cardiac function, nausea, vomiting, diarrhoea) within the 24-hour observation period. A moderate febrile reaction, increase in blood pressure and some swelling at the injection site have been observed. These symptoms significantly diminished or disappeared completely within the 24-hour observation period. 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 hours 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 TOLERIN administered subcutaneously at a dose of 4 ug/kg did not produce clinical symptoms (circulatory, respiratory, urinary, cardiac damage, nausea, diarrhoea) 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 hours after application. The laboratory tests performed at 24 hours did not show any alteration in blood coagulation, liver and kidney function. In the serum of treated individuals, taken at 24 hours, TNF was detected. There was also a moderate elevation of serum T_4 (thyroxin) in most individuals. The leukocyte count and the C3 complement fraction in the serum were also increased. The treated individuals were followed for three 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 (TOLERIN®)

After our initial clinical trials had been completed, the Hungarian National Agency of Public Health and Infectious Diseases gave permission for the trial of TOLERIN in 9 health institutions in both open and double-blind experiments. During these studies 110 surgical patients were treated with TOLERIN and 241 patients receiving similar medical treatment served as control. The majority of patients had been operated on for colon carcinoma. TOLERIN was given to patients 24^8 hours 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. 5 [52]. TOLERIN treatment stimulated bone marrow function, and leukocyte and granulocyte counts were increased significantly. This experiment indicates that TOLERIN may be used for the correction of bone marrow suppression caused by chemotherapeutic agents.

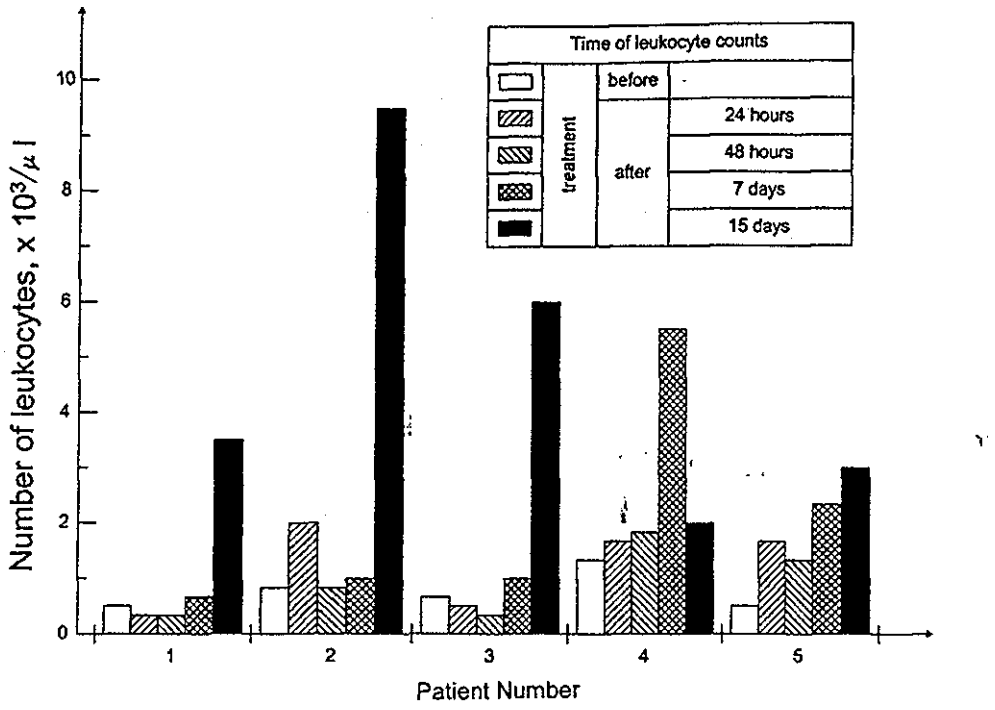

Figure 5. Effect of radiodetoxified endotoxin (RD-LPS: TOLERIN) on the number of leucocytes in CYSPLATIN treated patients bearing testis tumours.

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 year§ there was a significant clihical'improvement after TOLERIN treatment. This patient suffered from severe meningitis and encephalitis of unknown origin, Candida sepsis and mixed bacterial infections. The CD4+ and CD8+ T-cell count was 250 and 261, respectively. TOLERIN was given two times at 280 ug 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].

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