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APPLICATION OF PURIFIED POLYSACCHARIDES FROM CELL CULTURES OF THE PLANT *ECHINACEA PURPUREA* TO MICE MEDIATES PROTECTION AGAINST SYSTEMIC INFECTIONS WITH *LISTERIA MONOCYTOGENES* AND *CANDIDA ALBICANS*

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Abstract — Purified polysaccharides from cell cultures of the plant *Echinacea purpurea* were investigated for their ability to enhance phagocytes' activities regarding nonspecific immunity *in vitro* and *in vivo*. Macrophages (Mϕ) from different organ origin could be activated to produce IL-1, TNFα and IL-6, to produce elevated amounts of reactive oxygen intermediates and to inhibit growth of *Candida albicans in vitro*. Furthermore, *in vivo* the substances could induce increased proliferation of phagocytes in spleen and bone marrow and migration of granulocytes to the peripheral blood. These effects indeed resulted in excellent protection of mice against the consequences of lethal infections with one predominantly Mϕ dependent and one predominantly granulocyte dependent pathogen, *Listeria monocytogenes* and *C. albicans*, respectively. Specific immune responses to sheep red blood cells (antibody production) and to listeria (DTH) were not affected by the polysaccharides. The possibility of clinical use is discussed.

Treatment by natural remedies especially phytotherapy is becoming increasingly popular for patients and their doctors. Even though rough extracts from *Echinacea purpurea* plants have been used for more than a century for treatment of a variety of complaints no convincing studies about beneficial effects or mechanisms of actions exist. Satisfying results from experimental or clinical studies are not expected using such extracts because their composition is most variable. However, reproducible results could be obtained in a first approach using isolated polysaccharides, e.g. Stimpel, Proksch, Wagner & Lohmann-Matthes (1984); Wagner & Proksch (1985); Proksch & Wagner (1987); Lüttig, Steinmüller, Gifford, Wagner & Lohmann-Matthes (1989).

Such polysaccharides were purified from large-scale *in vitro* cultures of cells prepared from *E. purpurea* plants. They clearly exhibit immunomodulatory effects. This has been confirmed by screening tests such as *in vitro* and *in vivo* enhancement of phagocytosis (Wagner & Proksch, 1985; Wagner, Stuppner, Schäfer & Zenk, 1988) and by stimulation of macrophages (Mϕ) to secret mediators like TNFα, IL-1 and IL-6 (Lüttig *et al.*, 1989). Our study revealed further enhancement of microbicidal activities of Mϕ *in vitro* and of recruitment of phagocyte effector cells *in vivo* by the polysaccharides. Moreover, these substances mediated protection against the consequences of lethal infections with *Candida albicans* and *Listeria monocytogenes*.

Further experiments demonstrated effectivity of the polysaccharides in immunodeficient mice (after treatment with cyclophosphamide or cyclosporin A and after bone marrow transplantation). Some principal results from mouse experiments could be reproduced in human test subjects. These topics will be presented in two further papers. Furthermore, a clinical protocol for treatment of chronic infections with *C. albicans* has already been scheduled.

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EXPERIMENTAL PROCEDURES

Mice

Male 6–8-week-old balb/c and LPS resistant C3H/HeJ mice were received from Charles River (Würzburg, Sülzfeld, F.R.G.).

Chemicals and lymphokines

Escherichia coli lipopolysaccharide (LPS) was obtained from Difco Laboratories (Detroit, MI). Murine recombinant interferon-γ (IFN-γ) was produced by Genentech and supplied by Boehringer (Ingelheim, F.R.G.) and murine recombinant granulocyte–macrophage colony-stimulating factor (GM–CSF) was kindly provided by Behring Werke (Marburg, F.R.G.). Dextran sulphate was obtained from Sigma (Deisenhofen, F.R.G.).

Polysaccharides from cell cultures of the plant Echinacea purpurea

From the supernatant of *E. purpurea* cell cultures, three homogeneous polysaccharides have been isolated by DEAE–Sepharose CL-6B, DEAE–Trisacryl M and Sephacryl S400 column chromatography: two neutral fucogalactoxyloglucans with mean M_r of 10,000 and 25,000 and an acid arabinogalactan with a mean M_r of 75,000. The ratio of xyloglucans to arabinogalactan was approximately 2:1 in the polysaccharide batch used for all experiments. The substances have been further analysed by partial acidic and enzymic hydrolysis, methylation analysis and ^{13}C-NMR (for details see Wagner *et al.*, 1988). They have been screened for immunological activity by *in vitro* stimulation of granulocytes to enhanced phagocytosis, by stimulation of Mϕ to produce TNFα and by *in vivo* carbon clearance test. The polysaccharides used here were prepared as described (Wagner *et al.*, 1988) by Lomapharm (Emmerthal, F.R.G.). The limulus test, which is sensitive within 10 pg, was negative for LPS. The polysaccharides (up to 20 mg/ml) did not directly inhibit the growth of *Listeria monocytogenes* or *Candida albicans*, as revealed by a series of control experiments (six experiments for each condition).

Tumour cell line

J774A.1, a mouse monocyte–macrophage cell line had been adapted to culture from a tumour which arose in a female balb/c mouse in 1968 (ATCC TIB 67). It was maintained as a tissue culture in RPMI 1640 medium supplemented with 10% foetal calf serum (FCS) and 0.1% gentamicin. All components were purchased from GIBCO (Grand Island, NY).

Candida albicans

The *C. albicans* strain used throughout this study was isolated from a clinical specimen and identified by the taxonomic criteria of Lodder (1970). The yeasts were grown at 28°C under slight agitation in low-glucose Winge medium composed of 0.2% (wt/vol.) glucose and 0.3% (wt/vol.) yeast extract (BBL Microbiology Systems, Cockeysville, MD) until a stationary phase of growth was reached (about 24 h). Under these conditions, the cultures gave a yield of approximately 2.7×10^8 cells per ml, and the organism grew as an essentially pure yeast-phase population. After a 24 h subculture on Sabouraud-dextrose agar at 37°C, the cells were washed twice, diluted to the desired concentration, and used in the assays.

C. albicans *growth inhibition assay*

Mϕ monolayers (2×10^5 cells per well) were infected with different numbers of *C. albicans* microorganisms according to Decker, Lohmann-Matthes & Baccarini (1986).

Phagocytosis of yeast cells, as determined microscopically, was complete after 1 h. After a 12 h effector–target cell contact in the presence or absence of activating substances, plates were centrifuged (10 min at 800 g), the supernatant was removed, and lysis of the phagocytes in distilled water was performed and microscopically controlled.

After all effector cells had been lysed, serial dilutions were made in distilled water from each well. Plates (duplicate samples) were made on Sabouraud-dextrose agar with incubation at 37°C. Control cultures consisted of *C. albicans* incubated alone in complete medium (spontaneous growth) or, with identical results, *C. albicans* incubated with 2.5×10^5 thymocytes from untreated mice. Data were expressed as percent inhibition by effector cells by the following formula: % candidastatic activity = 100 – [(CFU expt group)/(CFU spontaneous growth)] × 100.

Determination of C. albicans *in spleen, liver and kidneys*

These organs of the infected animals were homogenized and the resulting suspensions were appropriately diluted in 0.9% NaCl. The number of colony-

forming units (CFU) was determined again by plate counting as described above.

Listeria monocytogenes (strain EGD) was kept virulent by continuous mouse passage. Cultures were obtained by growing a sample of spleen homogenate from an infected mouse in trypticase – soy broth. Bacterial suspensions were appropriately diluted in 0.9% NaCl and injected i.v. in a volume of 0.5 ml. The number of *L. monocytogenes* injected was confirmed by plate counting (e.g. Mackaness, 1962). At different time points after infection (as indicated) the spleen and the liver of the infected animals were homogenized and the resulting number of colony-forming units (CFU) was determined again by plate counting.

Listerial antigen for determination of the delayed type hypersensitivity (DTH) was produced according to Kaufmann, Simon & Hahn (1979). Listeria were cultured for three days in an ultrafiltrate (<10,000 mol. wt, Diaflo Hollow Fiber H1P10, Amicon, Lesington, MA) of trypticase – soy broth. The culture medium was centrifuged at 10,000 **g** and the supernate concentrated 200-fold (>10,000 mol. wt, Diaflo Hollow Fiber H1P10). After sterile filtration the concentrate was diluted in several 1:3 steps and tested for specificity by injecting 50 μl antigen solution into one hind footpad of immunized and untreated C57Bl/6 or DBA/2 mice. At a dilution of 1:81 footpad swelling took place in immunized mice only. Footpad thicknesses were measured 24 h after injection using a dial gauge caliper (Kröplin, Schlüchtern, F.R.G.) according to Hahn, Kaufmann, Miller & Mackeness (1979).

Preparation of cells from spleen, bone marrow and liver

Cell suspensions from spleen and bone marrow were obtained by standard techniques and deprived of erythrocytes by hypotonic lysis. Liver nonparenchymal cells (NPC) were obtained by collagenase digestion of the liver by the method used by Rich-man, Klingenstein, Richmann, Strober & Berzof-sky (1979). In brief, livers of pentobarbital-anaesthetized mice were blanched by perfusing 10 ml of HEPES-buffered Hank's balanced salt solution (pH 7.4) via the portal vein, followed by perfusion of 5 ml of 0.05% collagenase (Sigma, type IV, 300 U/mg) in Hank's balanced salt solution plus 5% FCS as a competitive inhibitor of nonspecific proteases. The perfused liver was excized, passed through a 50-gauge stainless-steel mesh, and then incubated for 45 min at 37°C in 40 ml of the collagenase solution used for perfusion.

Liver cells in suspension were then centrifuged for 3 min at 50 **g**, and the pellet, containing the bulk of hepatocytes, was discarded. The remaining cells were then washed twice in cold medium, and NPC were obtained by Percoll gradient centrifugation. One volume of Percoll (Pharmacia Fine Chemicals, Freiburg, F.R.G.) was mixed with 1 vol. of two-fold-concentrated RPMI 1640 to obtain a 50% Percoll solution. The resulting solution was diluted 1:1 with complete medium to obtain a 25% Percoll solution. A 5 ml amount of each step was then layered in a 15 ml plastic conical tube (Falcon Plastics, Oxnard, CA) and $50 \times 10^6 - 70 \times 10^6$ cells were placed on top of the gradient. The tubes were spun at 550 **g** for 30 min, NPC migrating between the two layers were collected and washed twice.

Liver macrophages (Kupffer cells) were obtained from the NPC suspension by selective adherence to plastic.

White blood count

Counting of white blood cells was performed according to standard techniques. Two 10 μl blood samples were taken from tail veins at different times after application of the polysaccharides in 0.5 ml NaCl 0.9% i.v. One sample was taken for erythrolyses and leucocyte count. The other was taken for a smear, stained according to "Pappenheim" (e.g. Rick, 1977) and differential counting. Controls were included to confirm that the leucocytosis was not merely due to repeated blood sampling and resulting stress for the animals.

Proliferation assay

^{3}H-d-Thymidine incorporation into replicating cells was measured to determine the proliferative capacity of different effector cell populations. A total of 5×10^4 cells/well in DMEM + 10% foetal calf serum were seeded into wells of flat-bottomed microtitre plates and incubated either in the presence or absence of GM-CSF (1000 U/ml) or 50 μl serum of pre-treated mice at 37°C for 72 h. One μCi/well of ^{3}H-d-Thd was then added, followed by another 18-h incubation period. The cells were then harvested by a Skatron suction filtration apparatus. The ^{3}H radioactivity bound to fibreglass filter paper was finally determined by β-scintillation counting.

Measurement of lucigenine dependent CL

A total of 2×10^5 J774-Mϕ cultured for 18 h either in the presence or absence of activating substances in HEPES-buffered RPMI plus 5% FCS were used for measurement of lucigenine dependent chemiluminescence (CL).

A 10 μl amount of lucigenine (bis-*N*-methyl-acridium nitrate, Sigma, Deisenhofen, F.R.G.) at

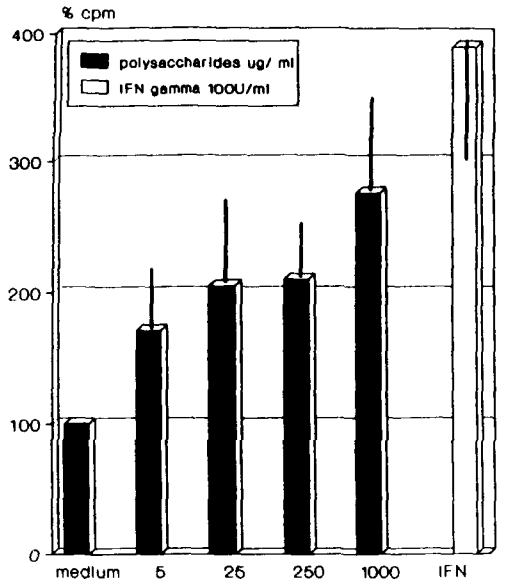

Fig. 1. ROI production by J774-Mϕ after incubation with polysaccharides or IFNγ. The six different preparations were always measured simultaneously. The ratio of counts from activated cells to those from the preparation containing medium only was calculated, $n = 6$, $P<0.005$ medium vs 1000 μg/ml of the polysaccharides.

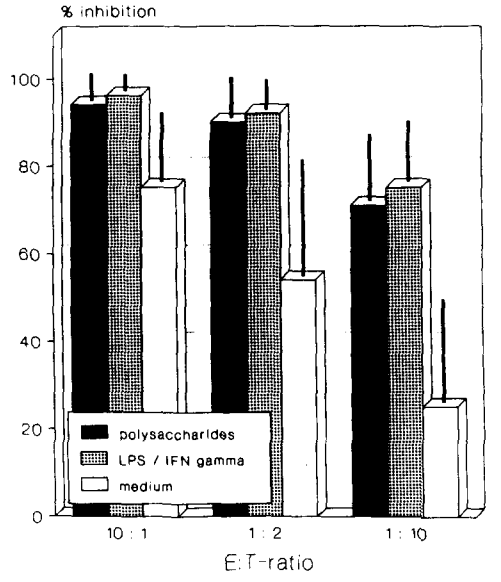

Fig. 2. Growth inhibition of *C. albicans* by activated Mϕ from the liver. The cells were preincubated for 18 h with the polysaccharides (0.2 mg/ml) or IFNγ (100 U/ml) and LPS (10 mg/ml), respectively, and then incubated with the yeast cells in numbers as indicated, $n = 5$, $P<0.01$ polysaccharides vs medium E:T = 1:10.

12 μM/l was added to 480 μl of the cell suspension according to D'Onofrio & Lohmann-Matthes (1984) and the background CL was monitored in a six-channel Berthold Biolumat (Berthold, Kubiziak, Ernst & Fischer, 1981). When background activity, always below 15,000 cpm, had reached constant values, 10 μl zymosan suspension from a stock solution (12.5 mg/ml) were added.

CL resulting from subsequent generation or reactive oxygen intermediates (ROI) was then measured for 30 min.

Software for computerized calculation of peak activities and integrals was supplied by Berthold (Wildbad, F.R.G.).

Statistical analysis

Standard deviations were calculated. The data were analysed by Wilcoxon's rank test. Differences were considered significant according to the *P*-values indicated.

RESULTS

Activation of Mϕ to produce TNFα, IL-1 and IL-6

The combination of three polysaccharides from cell cultures of the plant *E. purpurea* (see Experimental Procedures) has been chosen for analysis for two reasons. Firstly, production of single components on a large scale, as would be necessary for application to test subjects, has been almost impossible up to now. Secondly, the combination of the three components turned out to be more effective in screening tests (e.g. Wagner *et al.*, 1988). Therefore trials to get large amounts of single components were not stressed.

In a first step, experiments of Lüttig *et al.* (1989) using pure arabinogalactan were repeated, now using the polysaccharide mixture. All of the parameters tested paralleled the results described by Lüttig *et al.* (1989): thioglycolate induced Mϕ from the peritoneal cavity were induced to produce TNFα (control: <4 U/ml; polysaccharide 1.25 mg/ml: 4388 U/ml), IL-1 (control: 2.6 U/ml; polysaccharide 0.5 mg/ml: 9.1 U/ml) and IFN activity (predominantly IL-6 identical to IFNβ_2) in a dose-dependent matter (0.05 – 1.25 mg/ml polysaccharide; data not shown). Thus, the *in vitro* potential of the substances used here to activate Mϕ for secretion of these mediators was very similar to arabinogalactan.

In vitro *activation of Mϕ to enhanced microbicidal activity*

We now examined if the secretion of mediators by Mϕ was accompanied by enhanced microbicidal

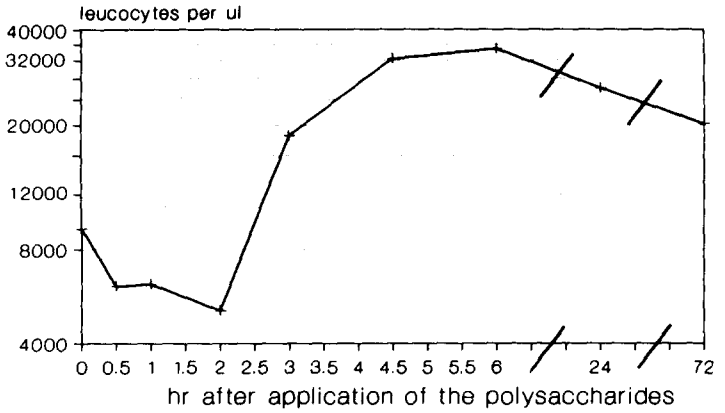

Fig. 3. White blood count of mice injected i.v. with the polysaccharides (0.2 mg). A representative result of five experiments is given.

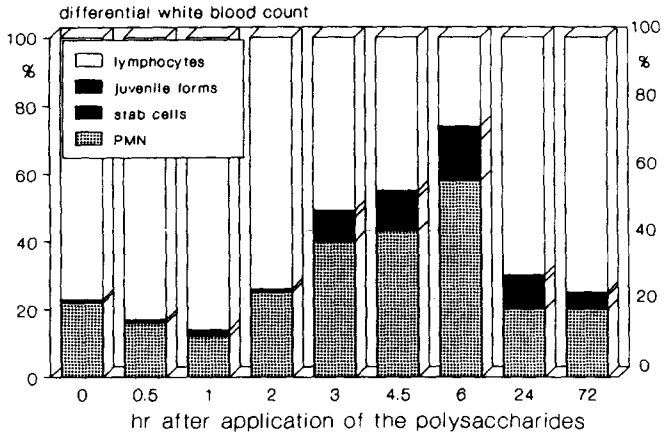

Fig. 4. Differential white blood count of the same samples as in Fig. 3.

activity of these cells. Therefore, J774 mouse Mϕ were incubated with the polysaccharides in different concentrations for 18 h. Their ability to produce reactive oxygen intermediates (ROI) was then measured after application of zymosan by lucigenine dependent chemiluminescence (Fig. 1). One mg/ml of the polysaccharides could enhance the ROI producing capacity of the J774 Mϕ by about 270% as compared to untreated cells. However, the polysaccharides were less effective than IFNγ (100 U/ml). Higher concentrations of the polysaccharides demonstrated the tendency to suppress ROI production (data not shown).

Previous experiments in our laboratory demonstrated that Mϕ from the liver (Kupffer cells) were more effective in killing yeast cells of the species *C. albicans* than Mϕ from the peritoneal cavity (Decker *et al.*, 1986) or J774 Mϕ. Therefore these Kupffer cells were used in the following *in vitro* test for growth suppression of *C. albicans* (Fig. 2). A quantity of 100 μg of the polysaccharides turned out to be as effective in activating the Mϕ to inhibit growth of *C. albicans* as the maximal activation by LPS and IFNγ together.

In vivo *effects of the polysaccharides on activities of phagocytes*

The polysaccharides induced a high increase, 3–4 h after i.v. application, in the number of peripheral blood leucocytes preceded by a slight, but reproducible fall (Fig. 3). Both the initial decrease and the following increase in the leucocyte number were due to changes in numbers of polymorphonuclear cells (PMN) as demonstrated by the differential blood count (Fig. 4). The absolute number of lymphocytes remained more or less constant. The initial

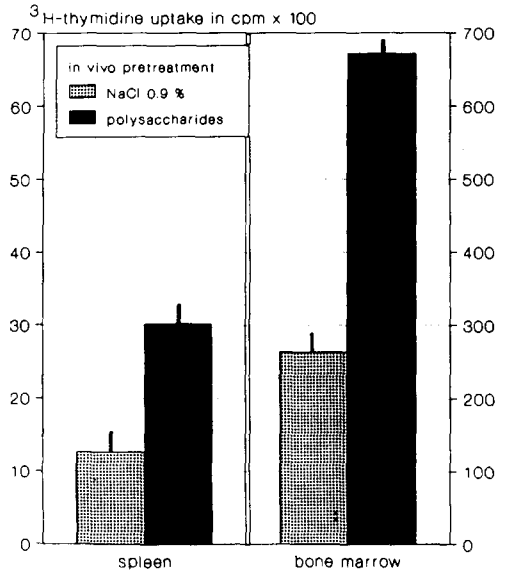

Fig. 5. Proliferative response of bone marrow and spleen cells to GM – CSF after *in vivo* pre-treatment with the polysaccharides. The mice were injected with 0.2 mg on three consecutive days. The cells were harvested 16 h after application of the last dose. A representative result of five experiments is given.

fall in the number of PMN may indicate their adherence to endothelial cells whereas the subsequent appearance of immature PMN indicates migration of cells from the bone marrow into the peripheral blood.

Moreover, the polysaccharides indirectly induce proliferation of bone marrow cells committed to the myeloid cell line. After *in vivo* treatment with the polysaccharides there was no visible splenomegaly, but the counts of the yield of white cells from the spleens were approximately 15% higher than in control mice.

In vitro proliferation of cells from bone marrow and spleen following incubation with murine recombinant GM – CSF was enhanced after *in vivo* application of the polysaccharides, indicating an elevated relative number of cells responding to the factor (Fig. 5).

The *in vivo* multiplication of phagocyte precursors could not be attributed directly to the effect of the polysaccharides, because they failed to induce bone marrow proliferation *in vitro* (Fig. 6). Probably the polysaccharides induced factors with colony-stimulating activity. This activity was indicated indirectly in the serum from *in vivo* treated animals by induction of proliferation of bone marrow cells (Fig. 6).

Enhancement of microbicidal activity of phagocytes in vivo

The resulting effects of the previous findings on infections with *L. monocytogenes* and *C. albicans* were further analysed because both infections depend to a great extent on the phagocytic activities in the early phase before T-cell mediated immunity is initiated.

With regard to nonspecific immunity Mϕ activities are crucial in natural resistance to listeria (Stevenson, Kongshvan & Skamene, 1981; Davies, 1983; Ohara, Mitsuyama, Miyata & Nomoto, 1985) whereas PMN endow the animals with high natural defence against candida (Lehrer & Cline, 1969; Rogers & Balish, 1977). But on the whole both kinds of phagocytes are proposed to be important for defence against both kinds of microbes.

Protection against systemic infection with L. monocytogenes

The polysaccharides could protect mice completely against the consequences of an LD_{80} infection dose of *L. monocytogenes* providing they were treated within a relatively short time interval with respect to the time point of infection (Fig. 7). The polysaccharides were without any significant effect if they were applied more then 18 h after the infection.

Their protecting effect in a dose-dependent manner could also be clearly demonstrated by considering the number of CFU of listeria in spleen and liver after different time intervals after infection (Figs 8 and 9).

The assumption that the protective effect of the polysaccharides was mediated mainly by Mϕ could be supported by the use of dextran sulphate as described by Hahn (1974). This substance destroyed Mϕ in spleen and liver as confirmed by nonspecific esterase staining and microscopic analysis. However, it induced an enhanced number of PMN 24 h after application. This kind of pre-treatment totally destroyed the protective effect of the polysaccharides against infection with listeria as confirmed by CFU counting in spleen and liver (data not shown). Therefore PMN alone, which was enhanced in number in this experiment, are probably not responsible for the protection.

Protection against systemic infection with C. albicans

The polysaccharides could protect 90% of mice against the fatal consequences of an LD_{80} infection dose of *C. albicans* in an experiment similar in design

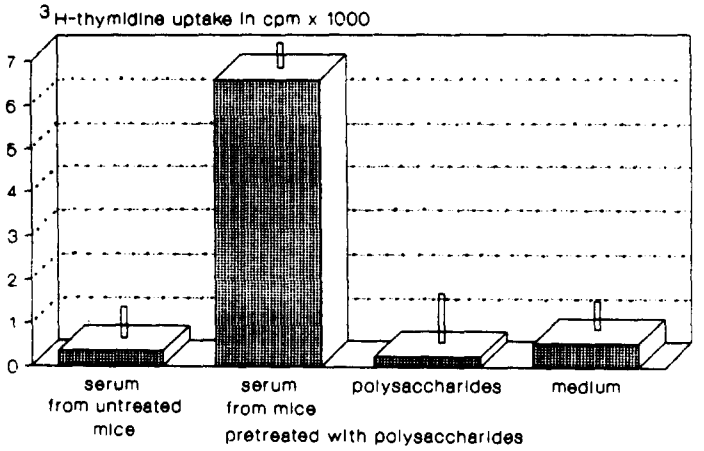

Fig. 6. Proliferative response of untreated bone marrow to the serum of mice pre-treated with the polysaccharides. The serum of the same mouse as in Fig. 5 was used giving a representative result of five experiments.

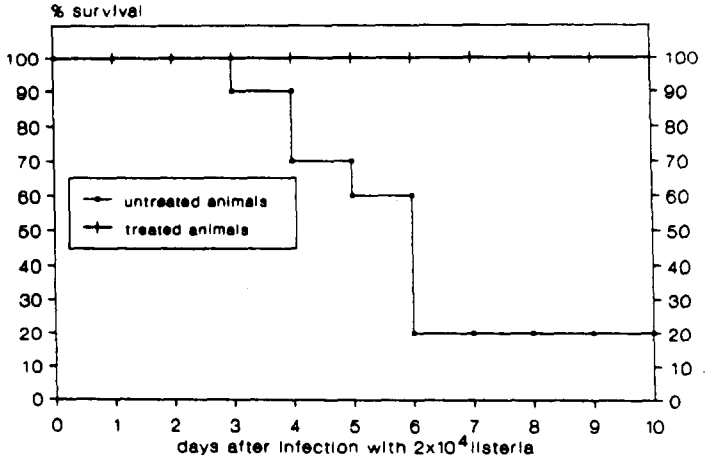

Fig. 7. Survival of treated vs untreated mice after infection with listeria. The polysaccharides (0.2 mg) were applied i.v. 24 h before, at the same time point with and 24 h after the infection. Twenty animals per group, $P<0.001$.

to that of Fig. 7 (data not shown). Again administration more than 18 h after the infection had less effect, if any. The number of CFU of candida in the kidneys was reduced to about 5% of the respective number of untreated animals (Fig. 10). Furthermore, no CFU were found in spleen and liver of treated animals, whereas 60% of untreated animals had few CFU in these organs.

Influence of the polysaccharides on specific immunity

No influence was found either on the production of antibodies against SRBC applied previously in different amounts or on DTH against listerial antigen. The animals had been infected with listeria in different sublethal doses from 10^1 to 10^3 and the DTH was monitored at different time points after infection as described by Kaufmann *et al.* (1979). Application of the polysaccharides at any time, simultaneously to the infection or simultaneously to the evaluation of the DTH, was without any effect (data not shown). Thus, initiation of specific immunity remains unaffected by the polysaccharides as tested so far.

Effects in LPS-resistant C3H/HeJ mice

The polysaccharides could induce a granulocytosis in C3H/HeJ mice including shift to the left, e.g. 7 h after i.v. injection of 1 mg of the substances, 20% lymphocytes, 55% PMN, 17% stab cells and 6%

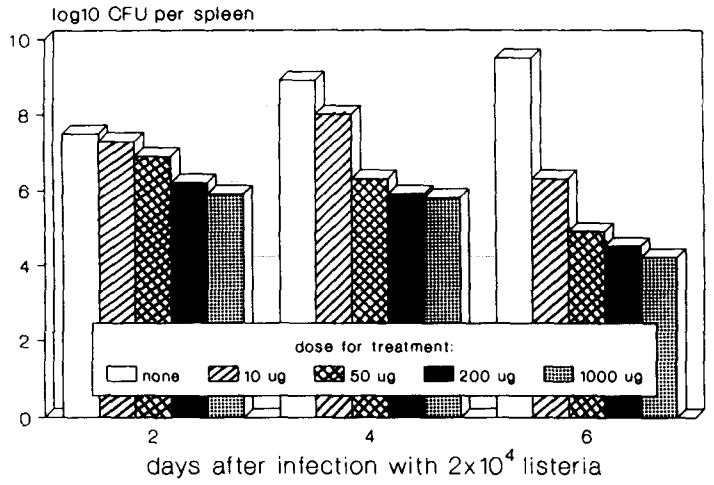

Fig. 8. Dose-dependent reduction of listerial CFU in the spleen of treated mice. The polysaccharides were applied i.v. at the time points as indicated in Fig. 7. Three animals per group except day 6: only one untreated mouse in a moribund state survived.

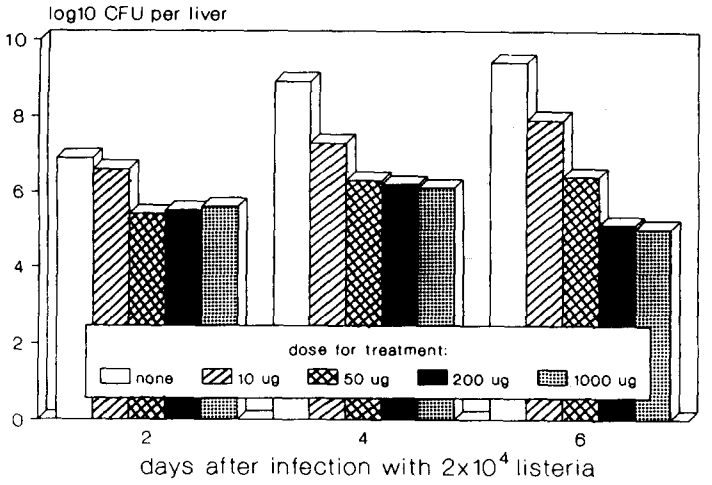

Fig. 9. Listerial CFU in the liver of the mice treated as indicated in Fig. 8.

juvenile forms were counted. Controls which received NaCl 0.9% or 20 ng LPS i.v. were included. Only a weak granulocytosis without a shift to the left was seen whereby no significant differences between the two kinds of treatment were observed. Furthermore, 24 h after treatment, PMN in the spleens and the liver NPC fractions were counted. Only in the case of polysaccharide application a drastic multiplication of PMN resulted (elevation from ca. 5% to ca. 20% in the spleen and from ca. 3% to ca. 30% in the liver NPC) whereas PMN of animals treated with NaCl or LPS were in the normal range (8% max.).

DISCUSSION

The polysaccharides from cell cultures of the plant *E. purpurea* could clearly enhance the phagocyte's activities *in vitro* and *in vivo*.

Mϕ from the peritoneal cavity could be stimulated to produce IL-1, TNFα and IFN activity. J774 Mϕ were activated to enhance production of ROI (Fig. 1). Kupffer cells demonstrated an increased ability to inhibit growth of *C. albicans* (Fig. 2). Proliferation of phagocyte-precursors in spleen and bone marrow (Fig. 5) and migration of PMN into

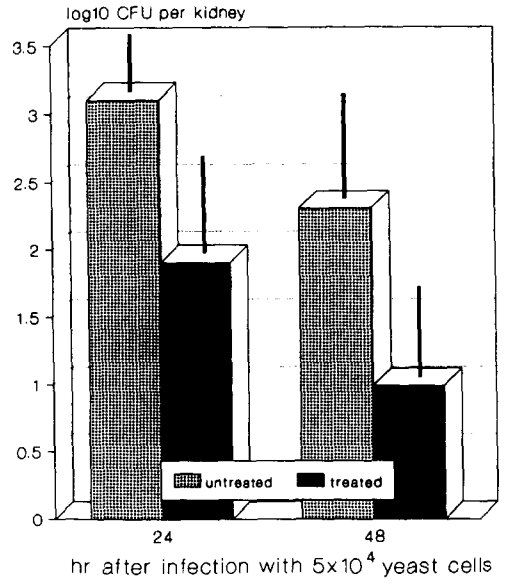

Fig. 10. CFU of *C. albicans* in the kidneys at different time points after i.v. infection of animals treated with the polysaccharides. Treatment as indicated in Fig. 7. Five animals per group, $P<0.02$ both time points.

the peripheral blood (Figs 3 and 4) was induced. These effects could indeed protect mice against lethal infections with *L. monocytogenes* (Fig. 7) or *C. albicans*. The protection was also reflected by reduced numbers of CFU of the agents in spleen (Fig. 8), liver (Fig. 9) and kidneys (Fig. 10), respectively. Specific immunity was not affected as examined so far.

This pattern of effects on the phagocyte system is very similar to effects resulting from application of small doses of LPS. Therefore, the first question which had to be answered was whether the effects were caused by contaminating LPS. The limulus test which was sensitive within 10 pg/ml and which had to be performed to satisfy the requirements of law regarding application to test subjects, was negative for LPS.

To further confirm that the results described here were not mediated by LPS we studied effects of the polysaccharides in LPS resistant C3H/HeJ mice. In these mice, too, these substances induced PMN whereas LPS was ineffective. This clearly indicates an action mechanism different from that of LPS.

As is well known, actions of phagocytes regarding nonspecific immunity can be divided into a sequence of steps, e.g. adherence, motility, phagocytosis, ROI production, etc.

Several of these functions could be enhanced by the polysaccharides *in vitro*: spontaneous motility of human PMN could be increased (in preparation). Therefore, migration towards a chemoattractant (f-MLP) was nonspecifically accelerated. Increased phagocytosis was found repeatedly (e.g. by Wagner *et al.*, 1985, 1988). ROI production by J774 Mϕ which is very important for the killing of microbes, could also be enhanced (Fig. 1). Finally, it could be shown that the activation of Mϕ by the polysaccharides really resulted in an increase of their microbicidal potential (Fig. 2). These results were applicable to *in vivo* test systems. The carbon clearance was increased by the polysaccharides (Wagner *et al.*, 1988) indicating activation of Mϕ in spleen and liver to enhanced phagocytosis. The initial fall in number of PMN in mice after injection of the polysaccharides (Figs 3 and 4) indicated activation of these cells to increase adherence to endothelial cells.

Furthermore, the number of phagocytes which can fight against invading microbes, was enhanced drastically (Figs 3 – 6). Increased migration from the bone marrow to the peripheral blood was demonstrated by the appearance of stab cells and juvenile forms (Fig. 4). Precursors of the myeloid cell line multiplied in spleen and bone marrow. This was demonstrated by enhanced *in vitro* proliferation to GM – CSF after application of the polysaccharides *in vivo*. This in turn indicated an increase in the number of myeloid precursors able to proliferate further *in vitro*.

Proliferation of myeloid cells was not directly induced by the polysaccharides as shown in Fig. 6, but proliferation inducing activity was found in the serum of treated mice. Probably, the polysaccharides induced production and secretion of CSFs. Macrophage activation, multiplication of precursors in different organs and migration to the sites of infection most probably contribute together to the good protection against infections with *L. monocytogenes* and *C. albicans* (Figs 7 – 10).

The assumption that the protection against listeria was mainly mediated by Mϕ could be supported by the application of dextran sulphate (Hahn, 1974). This substance eliminated Mϕ, but PMN were enhanced in number 24 h after the application, even though CFU in spleen and liver exceeded that of infected control animals and the protective effect of the polysaccharides was totally lost.

CFU of *C. albicans* in the kidneys of animals treated with dextran sulphate in the same way did not exceed that of controls. Therefore, the protection against this yeast is presumed to be mediated mainly by PMN.

Specific immune responses were not influenced by the polysaccharides as so far tested. For example,

infection with about ten listeria induced a slow developing weak DTH reaction. It was not affected by the polysaccharides either when applied simultaneously to the infection, or when applied together with the listerial antigen for DTH testing. This result is in accordance with Lüttig *et al.* (1989) and Stimpel *et al.* (1984) who could show that lymphocytes were not influenced by arabinogalactan *in vitro*.

On the whole, the pattern of inducing activities of phagocytes by the polysaccharides resembles the pattern induced by a first acute phase of infections, by toxins (LPS or others, even inorganic poisons) or by injuries. However, no toxicity could be found resulting from the polysaccharides (Lenk, 1989). Using the test conditions described, effects were limited to a relatively short time interval with respect to the time point of infection. This does not necessarily mean that the polysaccharides are ineffective in latent or chronic infections, but this issue remains to be tested. In any case, a beneficial effect in symptomatic acute severe infections appears rather unlikely because presumably the phagocyte system would already be maximally activated by the infection itself.

Of course, conclusions cannot be drawn to decide whether the polysaccharides from cell cultures of *E. purpurea* are suitable for clinical purposes merely from the results which are presented here. However, a clinical test protocol for treatment of chronic infections with *C. albicans* has already been scheduled.

Conclusions should also not yet be drawn on the effectivity of treatment or prophylaxis with marketable extracts from *E. purpurea* plants, since the most variable composition of components present in such extracts cannot be compared with the purified polysaccharide fraction used here.

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