Radioprotective and haemopoietic effects of some lipopolysaccharides from Rhodospirillaceae species in mice

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Summary. Lipopolysaccharides (LPS) from various *Rhodopseudomonas* and *Rhodospirillum* species were tested for their radioprotective efficiency against X-irradiation and for their influence on the growth of spleen colony forming units (CFU-s) in mice. The LPS from *Rhodopseudomonas gelatinosa* Dr_2 gave a high survival rate. It also favoured CFU-s formation and erythroid differentiation.

Bacterial lipopolysaccharides (LPS) are known to induce a variety of reactions in animals and humans[2,3]. Influences on haemopoiesis[4-7], on immuno-competent cells[8-10] and on radioprotective activity[11,12] have been reported. With respect to haemopoiesis the general view is that endotoxins and purified LPS favour myelopoietic differentiation[13-15]. We studied the influence of LPS from different *Rhodospirillum* and *Rhodopseudomonas* species on the development of spleen colony forming units (CFU-s) as well as their radioprotective efficiency. These lipopolysaccharides have lipid A types with different structures and biological activities[16].

Material and methods. Histocompatible young adult mice were used. They received acidified tap-water (pH 2.5-3.0) and a standard laboratory diet ad libitum.

Lipopolysaccharides from *Rhodospirillum tenue* 2761, *R. tenue* GFU, *Rhodopseudomonas viridis* F, *Rhodopseudomonas gelatinosa* 29/2, *R. gelatinosa* Dr_2 were prepared as described elsewhere[16]. Lipopolysaccharide from *Escherichia coli* 08:K27 was kindly provided by Dr K. Jann, Freiburg i.Br.

Male mice for the CFU-s assay[17] received 825 r whole-body X-irradiation followed some hours later by transfusion of $4 \cdot 10^4$ femoral bone marrow cells. The influence of individual LPS on the formation of CFU-s was tested by i.p. injection of each host animal with 25 µg LPS just before bone marrow transfusion. On day 8, half of the mice from each group received, respectively, either 1 µCi ^{59}Fe or 1 µCi ^{125}I-desoxyuridine ($^{125}IUdR$) approximately 3 h before sacrifice. Spleens and femora were removed. The spleens were fixed in Bouin's fluid overnight before weighing and scoring of surface CFU-s. The ^{59}Fe- and ^{125}I-activities in spleens and femora were measured in a crystal-scintillation counter.

The radioprotective efficiency (at 30-day survival) of each LPS was tested by injecting 10 µg LPS per mouse 24 h before exposure to 775 r X-rays at an exposure-rate of 75 r/min. Each test-group consisted of 50 female mice.

Results and discussion. The results are summarized in the table. The survival-rate for the LPS from *E. coli* 08:K27 is given for comparison (dose reduction factor 1.22 under equivalent conditions)[12]. As can be seen, the survival-rate with the LPS of *R. gelatinosa* Dr_2 is appreciably higher than that for *E. coli*.

Another striking feature of the LPS of *R. gelatinosa* Dr_2 was its influence on the proliferation of CFU-s. The CFU-s could not be scored because of confluent growth of the colonies. However, taking the mean spleen-weights as an index of cellular growth, it was observed that the spleens from this group were about 3 times greater in mass than those obtained for the other LPS studied. The uptake of ^{59}Fe and of $^{125}IUdR$ in the spleens and femora are regarded as reflecting the relative state of erythropoietic and proliferative activity, respectively. The group treated with LPS from *R. gelatinosa* Dr_2 showed significantly higher erythropoietic activity in both spleens and femora. On the other hand, a corresponding higher state of proliferation, indicated by $^{125}IUdR$-uptake, was not measured. This raises the question as to whether treatment with LPS from *R. gelatinosa* Dr_2 here favoured erythroid differentiation. The confluent surface spleen colonies may be in support of this, since surface CFU-s have been reported to be mainly erythroid in nature[18].

The confluence of colonies in this group occluded the possibility of discerning whether this condition was the result of a higher seeding of CFU-s or whether, at the same seeding efficiency, events leading to amplification of the erythroid line of differentiation were uniquely stimulated, resulting in exceptionally large colonies.

There is no direct proof for a correlation between the CFU-development and the radioprotective effect. Such correlations have, however, been shown for a number of radioprotective drugs using the endogenous CFU-s assay[19]. In contrast to the LPS of *R. gelatinosa* Dr_2, the LPS from *R. gelatinosa* 29/2 offered good radioprotection but gave no indication for a parallel influence on haemopoietic events. The LPS from the *R. gelatinosa* strains are highly toxic[15] as compared to that from *R. tenue* 2761 which is, relatively, less toxic. The results suggest that while only highly toxic LPS might have radioprotective capacities, not all toxic LPS influence haemopoietic events. Additional comparative data have to be collected to substantiate that.

Comparison of different assays after treatment of mice with various LPS. The rounded values represent means ± SD from the number N of animals

Assays	N	Control	*R. gelatinosa* Dr_2	*R. gelatinosa* 29/2	*R. viridis* F	*R. tenue* 2761	*R. tenue* GFU	*E. coli* 08:K27
Spleen-weight (mg)	6	24±3	75±12	22±4	27±4	19±5	21±3	25±3
CFU-s	6	6±2	confluent*	5±2	10±4	6±2	5±1	8±2
^{59}Fe in spleen (cpm)	3	2300±1000	31100±5400	1200±600	2700±2000	1200±300	1400±800	1600±700
^{59}Fe in femora (cpm)	3	2400±400	7800±1500	2300±200	2400±300	2600±200	2300±400	2400±800
$^{125}IUdR$ in spleen (cpm)	3	400±100	500±100	300±6	400±300	300±11	300±40	400±35
$^{125}IUdR$ in femora (cpm)	3	600±33	500±40	600±20	500±100	400±100	600±100	600±100
Survivals after r (%)	50	8	96	82	8	42	32	69**

* In this case the CFU-s could not be scored because of confluent growth.

** From Langendorff et al[12].

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2 E.H. Kass and S.M. Wolff, Bacterial Lipopolysaccharides: The Chemistry, Biology and Clinical Significance of Endotoxin. Univ. Chicago Press, Chicago & London 1973.
3 O. Lüderitz, C. Ganlanos, V. Lehmann, H. Mayer, E.T. Rietschel and J. Weckesser, Naturwissenschaften *65*, 578 (1978).
4 S.S. Boggs, P.A. Chervenick and D.R. Boggs, Blood *40*, 375 (1972).
5 O. Vos, Exp. Hemat. *6*, 688 (1978).
6 J. Gidali, I. Feher and V. Varteresz, Atomkernenergie *14*, 235 (1969).
7 A.F.G. Stevenson and J.-F. Duplan, Blut *32*, 361 (1976).
8 C.D. Baroni, G.S. de Franceschi and S. Uccini, Immunology *31*, 361 (1976).
9 R.A. Fox and K. Rajaraman, Cell. Immunol. *53*, 333 (1980).
10 U.H. Behling and A. Nowotny, Proc. Soc. exp. Biol. Med. *157*, 348 (1978).
11 W.W. Smith, I.M. Aldermann and R.E. Gillespie, Am. J. Physiol. *191*, 124 (1957).
12 H. Langendorff, M. Langendorff, K.H. Steinbach and J. Weckesser, Strahlentherapie *141*, 214 (1971).
13 P.J. Quesenberry, A. Morley, M. Miller, K. Rickard, D. Howard and F. Stohlmann, Blood *41*, 391 (1973).
14 T.J. MacVittie and R.I. Walker, Exp. Hemat. *6*, 613 (1978).
15 M.J. Koury, T.A. Kost, W.D. Hankins and S.B. Krantz, Proc. Soc. exp. Biol. Med. *162*, 275 (1979).
16 C. Galanos, J. Roppel, J. Weckesser, E.T. Rietschel and H. Mayer, Infect. Immunol. *16*, 407 (1977).
17 J.E. Till and E.A. McCulloch, Radiat. Res. *14*, 213 (1961).
18 J.L. Curry, J.J. Trentin and N. Wolf, J. exp. Med. *125*, 703 (1967).
19 K.E. Kinnamon, L.L. Ketterling, H.F. Stampfli and M.M. Grenan, Proc. Soc. exp. Biol. Med. *164*, 370 (1980).

Dopamine-containing cells in rabbit nodose ganglia

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Summary. Microspectrofluorometry of rabbit nodose ganglia exposed to formaldehyde vapor revealed that the intraganglionic fluorescent cells (SIF-cells) contain dopamine.

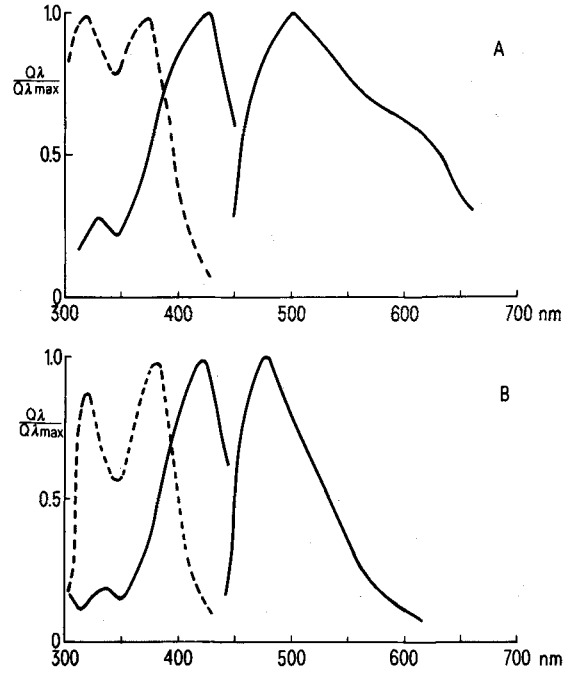
It has been reported that mammalian nodose ganglia contain small, intensely fluorescent cells (SIF-cells)[1-4], though the fluorescent monoamine in these cells has not yet been identified. The SIF-cells in mammalian sympathetic ganglia, on the other hand, have been studied by microspectrofluorometry[5] and shown to contain either a primary (dopamine or noradrenaline)[6-8] or a secondary catecholamine (adrenaline)[9]. The superior cervical ganglion of the rabbit, in particular, is well known to be endowed with SIF-cells characterized by dopamine fluorescence[10].

In the present investigation, the nodose ganglion of the rabbit has been studied using microspectrofluorometry and liquid chromatography to elucidate the biochemical characteristics of its SIF-cells in comparison with those of the superior cervical ganglion.

Nodose and superior cervical ganglia of 20 mature rabbits were used. Histochemical procedure; the ganglia were freeze-dried immediately after isolation and processed according to a minor modification of the Falck-Hillarp method[11] as described elsewhere[12]. The formaldehyde vapor treatment was performed, using paraformaldehyde stored at 75% humidity, at 80 °C for 30-60 min. Some freeze-dried ganglia were heated alone without exposure to formaldehyde vapor. The sections (10 µm thick) used for microspectrofluorometry were deparaffinized in xylene and exposed to concentrated HCl vapor for 1-3-10 min at room temperature according to Björklund et al.[5]. A Nikon fluorescence microscope (improved SPM-RFL system)[13] and a Zeiss fluorescence microscope (MPM01 system) were employed for measurements of excitation and emission spectra, respectively.

Biochemical determinations; the ganglia were freed from connective tissues and nerve trunks under a dissecting microscope, and stored at −80 °C. 14-20 nodose and 2 superior cervical ganglia were used for each determination. Catecholamine assay was made by a high-performance liquid chromatograph (Yanagimoto, L-2000L) with a high-sensitivity electrochemical detector (Yanagimoto, VMD-101). The practical assay procedure followed the method of Refshauge et al.[14] modified by Kojima et al.[15].

The chemical analyses showed that the nodose and superior cervical ganglia contained both dopamine and noradrenaline. Dopamine amounted to 51 ± 13 ng/g in the nodose



Excitation and emission spectra from SIF-cells of rabbit ganglia. *A* Nodose ganglion, *B* superior cervical ganglion. ———, Before exposure to HCl vapor; ----, after exposure to HCl vapor for 10 min.