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BIOLOGICAL PROPERTIES OF SURFACE COMPONENTS OF *LISTERIA MONOCYTOGENES* (E_i FACTOR)

F. PATOČKA, M. MÁRA, B. POTUŽNÍKOVÁ, A. JIRÁSEK, E. MENČÍKOVÁ

Laboratory for Special Medical Microbiology, 1st Department of Pathology and Department of Medical Microbiology and Immunology, Charles University Medical Faculty, Prague, Czechoslovakia

We have studied the problem of *Listeria monocytogenes* fifteen years ago and we have again approached this problem because despite the newest findings in this field the pathogenicity of *L. monocytogenes* is not yet fully explained. We would like to recall some of the stepping stones, so to say, along the uneasy path of discovery:there was the not fully appreciated MPA factor (Stanley, 1949), there was listeriolysin an undoubtedly active exotoxin (Njoku-Obi et al., 1963, Jenkins et al., 1964), then the NAD-ase activity of partially purified preparations associated with hemolytic activity, the "platelet-damaging factor", the cellulolytic activity (Siddique et al., 1970). Although much has been accomplished already the work is not complete.

In our studies of factors conditioning the pathogenicity of *L. monocytogenes* our investigations were intentionally focused on two strains of this microbe: K_1, causing only a light hemolysis beneath its colonies in rabbit blood agar plates with no measurable titer of hemolysin, and strain K_2, causing distinct hemolysis in rabbit blood agar plates and producing hemolysin in titers demonstrable in vitro. Table 1 shows the serotype, titre of hemolysin, and LD_{50} for mice of these strains together with those of a nonvirulent strain K_3 isolated by Welshimer.

The isolation and composition of factor E_i was just described by Dr. Mára. In conventional rabbits intradermal administration of 0,3 ml of this factor causes a distinct macroscopic inflammatory reaction the first signs of which appear in six to twelve hours, its maximum develops in 24 to 48 hours and then begins to recede. This reaction consist of erythema and oedema. Rarely signs of apical necrosis or hemorrhage appear. After the picture of acute inflammation recedes a palpable nodular reaction persists. A reaction is apparent even after applica-

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tion of 0,02 mg of E_i, which is the *minimal reacting dose* (MRD). Preparations from either strain, K_1 or K_2, cause reactions of identical nature macroscopically.

Histological examination revealed in the skin inflammatory oedema or phlegmona with subcorneal pustules in the epidermis and in some cases the phlegmonous changes reached into subcutaneous regions, and inflammation affected muscles and altered blood vessels (Fig. 1). This reaction is apparent after six hours already. Quantitatively the same reaction is caused by E_i prepared from cell walls (Fig. 2). A substantially weaker reaction is caused by phe-

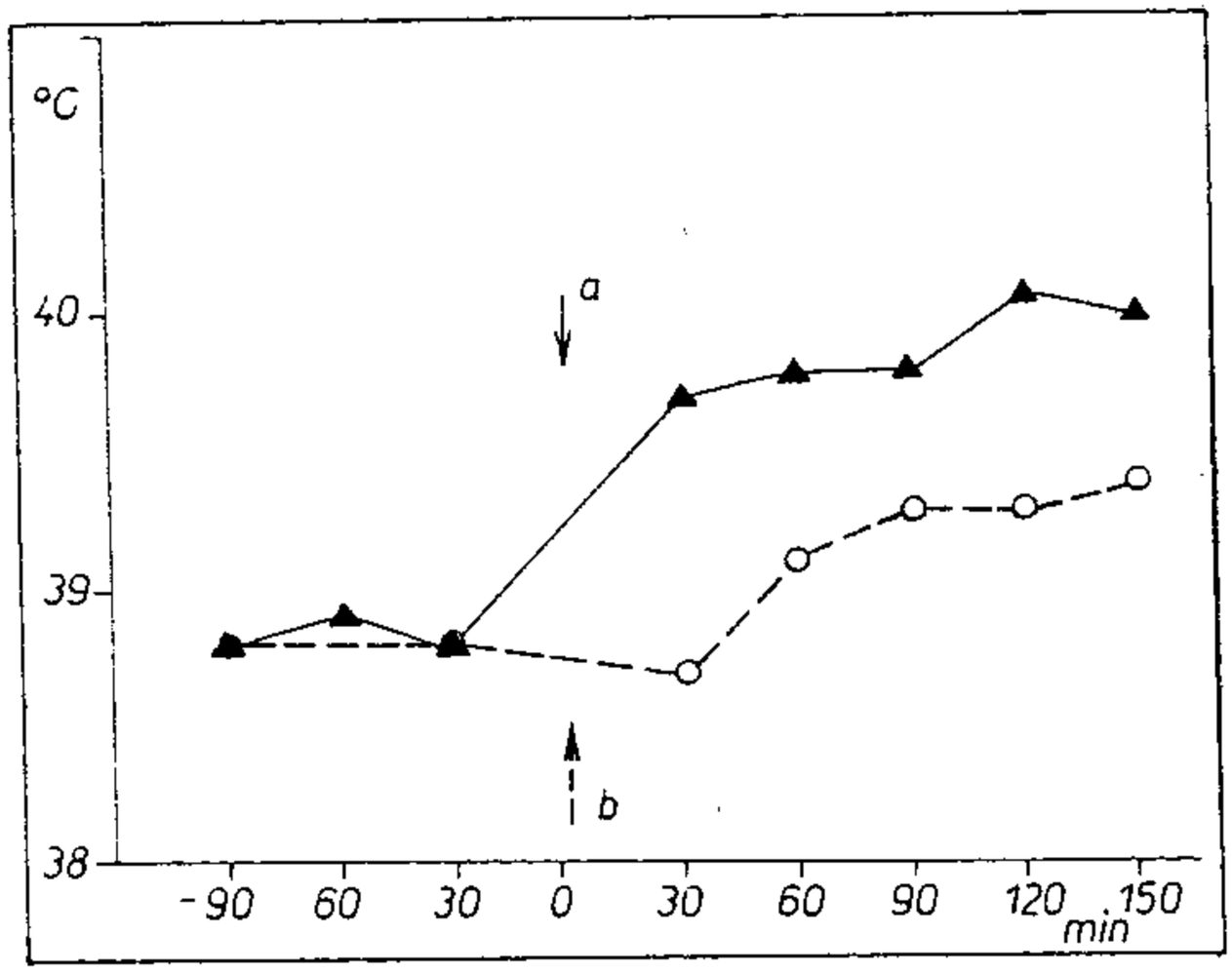

Fig. 4. Pyrogenity of E_i. a — application 11.5 mg E_i i. v.; b — 2.5 ml phosph. buffer i. v.

nolic extract of listerial cells and a hardly discernible reaction is caused by TCA extract and an aqueous extract obtained from residues of bacterial cells after extraction of E_i by ether.

Doses of up to 15 mg of E_i administered intravenously to rabbits were not lethal. Histological examination of organs of animals sacrificed in various intervals after intravenous application of E_i did not reveal any changes.

E_i alone did not elicit any of the Shwartzman reaction, however, when E_i was used for sensitization and endotoxin of *E. coli* was then given intravenously a marked local Shwartzman reaction was elicited (Fig. 3).

In rabbits E_i in a dose of 4,5 mg per kilogram given intravenously is pyrogenic. Fig. 4 shows temperature averages of three treated and three control animals.

The antigenicity of E_i was tested by immunizing rabbit (serum no. 1) partly with killed cells of listeriae and live bacteria, and partly with E_i in incomplete Freund's adjuvant (serum no. 4). For control purposes a commercial agglutination serum against *L. monocytogenes* O antigen type I was used. These sera precipitated with E_i factor of strain K_1 in Ouchterlony's diffusion test. Maximum of precipitation in four zones was attained with serum no. 3 which was obtained from rabbits immunized with E_i in complete Freund's adjuvant.

Sheep erythrocytes sensitized with E_i factor alone were agglutinated by serum no. 4 in titers up to 1:2560. Passive hemagglutination values attained with the ether sera were much lower.

Table 1

L.m. strains	Serotype	LD_{50} for mouse i.p.	Haemolysin 2.5% sheep erythr.
India	1	7.8×10^6	0
Brat 1 (K_1)	1	2.6×10^6	0
Brat 2 (K_2)	5	3.3×10^8	1/40
Brat 3 (K_3)	4	avirulent	0

Neutralization of E_i factor's dermal reaction in the rabbit in vitro was negative. Therefore a rabbit hyperimmunized with live and killed listeriae was tested with E_i. At the site of injection a conspicious local inflammatory reaction with oedema developed which was substantially greater than the already described reaction. This proved than E_i also acts as an allergen.

Due to this observation serological and bacteriological tests were performed in our conventional animals. It was found that most of them had a nonspecific agglutination titer against *L. monocytogenes* O antigen type I (1:20 to 1:40). Similar nonspecific titers were observed on passive hemagglutination of sheep erythrocytes sensitized with E_i factor. Bacteriological examination for listeriae of the animals' feces, organs and mucous membranes, however, were negative. The intensity of the intradermal reaction of E_i, that is the determination of MRD, was not corelated with these serological findings.

To prove that the dermal reactions in conventional animals are due to the primary toxicity of our preparations we performed in collaboration with Dr. Štěpánová of the gnotobiological department at the Institute of Microbiology of the Czechoslovac Academy of Sciences two series of experiments in three month old gnotobiotic rabbits. The macroscopic picture and histological findings in these animals were the same as in conventional animals.

In conventional and inbred mice no lethal effect was observed after administration of E_i factor.

On the other hand mice treated with sublethal doses of Actinomycin D [12,5 μg per mouse according to Pieroni (1970)] succumbed to doses of 10^1 μg of E_i within 7 days. The toxic effect of E_i inactinomycin D — treated mice can be neutralized by anti-E_i hyperimmune serum no. 4 (Patočka, Mára 1973).

In the last series of experiments we tried to verify whether our observations made in 1958 and confirmed by Silverman in 1961 are valid for E_i factor. It was found that this factor significantly lowers the LD_{50} of the virulent strain K_1 of

Table 2. Enhancing effect of E_i on listeria monocytogenes infection

	Infection only	Infection E_i isolated from K_1	Infection E_i isolated from K_3
LD_{50} strain K_1	7.2×10^6	1.2×10^5 index 60	2.1×10^4 index 342
LD_{50} strain K_3	0	0	0

Table 3. Prevention of Listeria monocytogenes infection by E_i factor

Days of application of E_i before infection	Number of mice	Number of deaths
3	5	1
2	5	0
1	5	1
0	5	5
infection only	5	4

L. monocytogenes Table 2. The table also shows that an analogous preparation obtained from Welshimer's nonvirulent strain (K3) has a similar synergistically virulence potentiating effect. On the other hand both preparations administered simultaneously have no effect on the nonvirulent strain K3.

When a lower dose (100 μg per mouse) of E_i was administered 1 to 3 days before infeotion its lethal effect was prevented (Tab. 3).

The described biological activities (in so far as they has been compared) are practically the same in preparations of E_i isolated from strains K1 and K2 (Mára et al. 1974).

From what has been presented in both of our communications we dare conclude that in view of the mode of preparation of E_i factor from cell walls of *L. monocytogenes,* the so far determined chemical constitution of the factor composed of proteins, polysaccharides and freely- and firmly-bound lipids, and some biological properties, such as pyrogenicity, reactivity in the skin of rabbits, lethal effect in Actinomycin D — treated mice and the prevention of lethal listerial infection in mice, the E_i preparation isolated by us is suggestive of the properties of endotoxin of Gram-negative bacteria.

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F. Patočka, Laboratory for Special Medical Microbiology,
Studničkova 7, Prague 2, Czechoslovakia