STIMULATION OF NONSPECIFIC RESISTANCE OF MICE BY HIGH- AND LOW-MOLECULAR-WEIGHT FRACTIONS OF ENDOTOXIN IN TWO MODELS OF *Shigella* INFECTION

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The ability of the endotoxin of *Salmonella paratyphi* B and of its high-(HMF) and low-molecular-weight (LMF) fractions to induce resistance in mice to *Shigella* infection by the intraperitoneal and intranasal route was investigated. After intraperitoneal infection HMF possessed high activity (index of efficacy — IE = 9.5), much higher than the activity of the original preparation (IE = 5.8) and of LMF = (IE = 2.8), and on the lung model all preparations were less active (IE between 1.6 and 1.7). The results suggest that during the study of the phenomenon of induction of nonspecific resistance, separate attention must be paid to conditions leading to antitoxic and antiinfectious resistance.

KEY WORDS: *nonspecific resistance; high-molecular-weight and low-molecular-weight fractions of endotoxin; models of* **Shigella** *infection.*

Specific activity of the endotoxin of the enterobacteria is localized chiefly in the high-molecular-weight fraction [9], and their ability to induce nonspecific resistance to bacterial toxins is characteristic of both the high- (HMF) and low-molecular-weight (LMF) fractions [10]. The level of nonspecific resistance to various experimental infections is known to depend on the character of the infectious process. In mice, following intraperitoneal injection of *Escherichia coli* and of dysentery and typhoid bacteria a toxico-septicemic process develops, which is far different from the disease in man [11]. Preliminary injection of endotoxin in this case leads to the development of marked nonspecific resistance [2]. However, the level of protection against *Salmonella typhimurium*, naturally pathogenic for mice, is low [2, 13].

In the investigation described below the activity of HMF and LMF from an antigenic preparation of *S. paratyphi* B in relation to the induction of nonspecific resistance was studied on two models of experimental *Shigella* infection in mice: Intraperitoneal and intranasal infection. By contrast with intraperitoneal infection, intranasal infection with dysentery bacteria led to the cyclic development of an isolated focus in the lung [5]. In this case the infectious process was pathogenetically similar to the process arising in monkeys with this disease [6].

EXPERIMENTAL METHODS

The original preparation of endotoxin was obtained by the method of tryptic proteolysis from a submerged culture of *S. paratyphi* B 42 [14]; HMF and LMF were separated by fractionation of the endotoxin on Sepharose 2B columns [3]. The molecular weight of HMF and LMF was $1 \cdot 10^6$-$10 \cdot 10^6$ and $10 \cdot 10^3$, respectively [4]. The content of anthrone-positive carbohydrates, heptones, 2-keto-3-deoxyoctonate, nucleic acids, and protein was determined in the original preparation and in HMF and LMF. Stimulation of nonspecific resistance to infection was determined in noninbred albino mice weighing 16-18 g. The doses of the preparations injected intravenously did not exceed 0.25 LD_{50}, and for the original preparation they were 12.5 and 50 μg, for HMF 12.5 μg, and for LMF 12.5 and 500 μg. After 24 h, a culture of *Shigella sonnei* 177^b was injected intraperitoneally into the animals of one group in doses of 200, 100, 50, 25, 12.5, and 6.25 million bacterial cells, and the same culture was injected intranasally

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TABLE 1. Intensity of Nonspecific Resistance Induced by Endotoxin Preparations on Two Models of *Shigella* Infection

Preparation	Dose of toxin, μg	Mode of infection	Infecting dose of Shigella culture, millions of bacterial cells										LD_{50} and its confidence intervals, millions of bacterial cells	IE
			200	100	50	25	12,5	6,25	3,12	1,56	0,78	0,39		
Original	12,5	Intraperitoneal	9/10	6/10	4/10	0/10	0/10	0/10	—	—	—	—	75,6 57,3—99,8	5,8
	50		—	2/10	1/10	0/10	0/10	0/10	—	—	—	—	115,0 108,4—283,1	8,8
HMF	12,5		9/16	4/16	5/16	0/16	1/16	0/16	—	—	—	—	124,2 97,5—163,9	9,5
LMF	12,5		15/15	14/16	10/16	6/16	1/16	1/16	—	—	—	—	36,9 28,0—48,7	2,8
	500		11/16	9/16	2/16	0/16	0/16	0/16	—	—	—	—	109,1 82,7—144,1	8,4
Physiological saline	—		16/16	16/16	14/16	12/16	9/16	4/16	—	—	—	—	13,1 9,9—17,2	—
HMF	12,5	Intranasal	—	—	—	—	16/16	16/16	15/16	10/16	1/16	2/16	1,15 0,9—1,5	1,6
	12,5		—	—	—	—	16/16	15/16	13/16	6/16	4/16	1/16	1,25 0,9—1,6	1,7
	500		—	—	—	—	6/6	6/6	6/6	3/6	1/6	0/6	1,22 0,9—1,6	1,7
Physiological saline	—		—	—	—	—	16/16	14/16	16/16	15/16	5/16	5/16	0,72 0,5—1,0	—

Note. Numerator gives number of dying mice; denominator total number of mice.

into the animals of the other group in doses of 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 million bacterial cells. The results were read 10 days after infection, with determination of the index of efficacy (IE): the ratio between the minimal lethal dose of the culture causing death of 50% of the animals in the experimental and control groups. LD_{50} was calculated by the Kärber–Ashmarin method [1].

EXPERIMENTAL RESULTS

The original preparation and HMF and LMF stimulated the resistance of the mice to intraperitoneal infection with a culture of *Shigella sonnei* (Table 1). After injection of equal doses of the preparations (12.5 μg) HMF gave the greatest protective effect (IE = 9.5). The original preparation was less active (IE = 5.8). LMF had the least activity (IE = 2.8). With an increase in the dose of the original preparation by 4 times (50 μg) and LMF by 40 times (500 μg) the protective effect equal to that of HMF could be reproduced.

The higher the molecular weight of the preparation and the higher its content of polysaccharide components of the basic structure of endotoxin, namely hexoses, 2-keto-3-deoxyoctonate, and heptoses, in it, the stronger its ability to stimulate nonspecific resistance (Table 2). Some of the activity of LMF can probably be explained on the grounds that it contained low-molecular-weight fragments of nucleic acids (poly- and oligonucleotides), which are themselves stimulators of nonspecific resistance [12]. Another possibility is that the development of resistance in this case may be connected with other mechanisms [10].

In the experiments with the lung model the protective action of these preparations was much weaker. Intravenous injection of all tested doses of HMF and LMF led to an increase in LD_{50} of the *Shigella sonnei* culture on intranasal infection by only 1.6-1.7 times (Table 1). After intranasal infection of mice, the pathogenic agent is known to proliferate intensively

TABLE 2. Chemical Composition of Original Endotoxin and of Its HMF and LMF (in %; M±m)

Endotoxin	Anthrone-positive carbohydrates (hexoses)	Heptoses	2-keto-3-deoxy-octonate	Nucleic acids	Protein
Original	28,9±0,6	0,4±0,01	2,68±0,19	9,36±1,1	18,1±1,25
HMF	39,6±0,6	1,6±0,12	4,99±0,02	1,73±0,11	16,4±0,2
LMF	18,3±1,3	0	0,88±0,02	17,8±2,77	16,8±0,75

in the lung tissue. Whereas after intraperitoneal infection suppression of the process is probably due to an increase in antiendotoxic resistance [7-8], in a developed infectious process following a progressive course (lung model) this stimulation was evidently less effective.

When induction of nonspecific resistance is studied, separate attention must therefore be paid to the conditions responsible for antitoxic and antiinfectious resistance. The ability of endotoxins to stimulate nonspecific resistance is determined by their high-molecular-weight component.

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EFFECT OF INFLUENZA VIRUS AND ITS STRUCTURAL COMPONENTS ON THE IMMUNOCOMPETENT SYSTEM OF ANIMALS

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The effect of influenza A ($PR_8/34$) virus and its structural components on immunologic reactivity was studied in mice. Neuraminidase, the enzyme of the influenza virus outer membrane, possesses an immunodepressive action. The addition of neuraminidase leads to removal of sialic acids from the surface of the lymphocytes and reduces their electrophoretic mobility. The mechanism of the immunodepressive action of neuraminidase is discussed.

KEY WORDS: *influenza virus; immunodepression; neuraminidase; lymphocytes.*

The pathogenesis of influenza infection and the complications accompanying it have not yet been properly explained. In recent years immunologic changes in influenza have been

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