WEAKENING THE BACTERICIDAL ACTIVITY OF MOUSE PERITONEAL MACROPHAGES BY A COMBINATION OF TYPE A STAPHYLOCOCCAL ENTEROTOXIN AND ENDOTOXIN

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UDC 616.155.33-008.13-008.64-02:[615.919:579.861.2

KEY WORDS: peritoneal macrophages; type A staphylococcal enterotoxin; endotoxin; bactericidal activity

The mechanisms of the pathogenic action of staphylococcal enterotoxins (SE) have not been adequately studied. Blockade of the reticuloendothelial system (RES) by thorotrast is known to increase the sensitivity of animals to SE-induced vomiting [4]. This suggests that the functional status of the RES plays an important role in the response of the body to enterotoxin. Data in the literature are evidence that SE may also influence the function of phagocytic cells. First, administration of SE to monkeys via a gastric tube leads to the development of acute gastroenteritis with exudation of neutrophils, macrophages, and with other signs of inflammation [3]. Second, a very important property of SE is its ability to sensitize animals to the lethal action of endotoxins of Gram-negative bacteria (lipopolysaccharide – LPS), which places them in the same category as substances causing hyperactivation of the RES, and also exerting a sensitizing action [5]. Considering the constant contact of the body with conventionally pathogenic bacteria and, correspondingly, with endotoxins of the intestinal microflora, the investigation of macrophagal functions responsible for the elimination of microorganisms during the action of SE and in response to their combined administration with LPS, becomes a particularly urgent problem. The aim of the present investigation was accordingly to study the basic principles of changes in the phagocytic and bactericidal functions of macrophages under the influence of type A SE (ASE) and LPS.

TABLE 1. Total Number of Cells in Mouse Peritoneal Exudate 2 and 24 h after Injection of ASE and LPS

Agent administered	Number of cells, $\times 10^6$/mouse	
	2 h	24 h
PBS	3,34±0,46	3,85±0,23
ASE (1 μg per mouse)	1,7±0,15*	1,48±0,6*
LPS (20 μg per mouse)	—	6,8±1,2*
ASE +LPS	—	1,9±0,6*

Legend. Here and in remaining tables: PBS) phosphate-buffered saline; doses of ASE and LPS when given together were similar to those when given separately; *$p < 0.01$, **$P < 0.05$ compared with control (PBS).

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 0007-4888/89/0011-1612$12.50

TABLE 2. Change in Phagocytic Activity of Macrophages of Mice Treated with ASE and LPS

Agent administered	Index of phagocytosis, % of control	
	2 h	24 h
PBS	42,4±3,4 (100)	39,1±0,8 (100)
ASE	25,6±0,5* (60)	53,9±2,1 (137)*
LPS	—	60,8±3,6 (155)*
ASE + LPS	—	49,3±5,3 (126)**

Legend. Doses in Tables 2 and 3 similar to those in Table 1.

TABLE 3. Changes in Bactericidal Activity of Macrophages of Mice Treated with ASE and LPS

Agent administered	Index of completion of phagocytosis, % of control	
	2h	24h
PBS	64,2±3,5 (100)	61,4±1,68* (100)
ASE	31,1±3,2* (48)	90,1±1,5* (146)
LPS	—	90,6±2,6* (147)
ASE + LPS	—	17,3±2,8* (28)

EXPERIMENTAL METHOD

Experiments were carried out on male C57BL/6 mice weighing 16-18 g. Animals of each group (four in each case) received an intraperitoneal injection of isotonic phosphate-buffered saline, pH 7.2, ASE (Ufa), and prodigiosan (Serratia marcescens endotoxin), 4 h after administration of endotoxin, as described previously [2]. The mice were killed 2 and 24 h after the injection, their peritoneal cells were isolated [7], and their concentration adjusted to 1×10^6 cells/ml. For working with the cell cultures, medium RPMI-1640 with glutamine ("Flow Laboratories"), 25 mM HEPES ("Serva"), and 10% embryonic calf serum ("Serva") was used. The ability of the cells to ingest and kill Staphylococcus aureus DO 318 was determined in a 24-h culture of macrophages [1, 7]. Phagocytic activity was evaluated by determining the decrease in the number of intracellular microorganisms in medium with macrophages compared with cell-free medium during incubation for 1 h, and expressed as the decrease in the initial number of viable bacteria, expressed as a percentage. The phagocytic index was calculated by the formula:

$$\frac{N_0 - N_{60'}}{N_0} \cdot 100,$$

where N denotes the number of viable extracellular bacteria. Completion of phagocytosis was determined by the method in [1], the only difference being that macrophages in the monolayer were lysed by a 0.05% solution of sodium deoxycholate. The index of completion of phagocytosis was calculated in the same way:

$$\frac{N_0 - N_{60'}}{N} \cdot 100,$$

where N denotes the number of viable intracellular bacteria.

EXPERIMENTAL RESULTS

The experiments of series I to study phagocytic and bactericidal activity were undertaken with macrophages obtained from mice of the following groups: 1) 2 h after injection of enterotoxin; 2 and 3) 24 h after administration of ASE and LPS separately, 4) 24 h after administration of endotoxin preceded by ASE. The results of determination of the total number of peritoneal cells in all the groups studied are shown in Table 1. ASE caused a 50% decrease

TABLE 4. Effect of ASE and LPS on Macrophagal Function in Vitro

Agent administered	ASE (1 μg per mouse)	Index of completion of phagocytosis
PBS	38,4±2,2 (100)	62,9±6,8 (100)
ASE (1 μg per mouse)	45,5±4,2 (118)	88,3±5,6 (140)*
LPS (20 μg per mouse)	52,6±5,5 (136)**	85,7±1,3 (136)*
ASE + LPS (a)	40,9±0,1 (106)	82,4±5,4 (131)**
ASE + LPS (b)	36,8±2,1 (95)	34,2±1,2 (54)*

Legend. a) LPS added to incubation medium simultaneously with ASE, b) 4 h after ASE.

in the total number of cells as early as 2 h after injection; after 24 h the total number of cells still remained depressed. Whereas prodigiosan increased the yield of cells in the peritoneal cavity of intact mice, in mice treated with ASE not only did their number not increase under the influence of LPS, but it was actually significantly lower than in the control.

The study of the phagocytic and bactericidal activity of the macrophages 2 h after administration of ASE to the mice revealed a marked decrease compared with values of these parameters in the control animals (Tables 2 and 3). Stimulation of phagocytosis was observed 24 h after injection of ASE and LPS separately. These patterns of change in the phagocytic function of the macrophages thus revealed are in complete agreement with data in the literature obtained by the study of carbon clearance in rabbits after injection of staphylococcal enterotoxins. Sugiyama [6] also observed biphasic changes in the phagocytic function of the RES; depression of the degree of carbon clearance 2 h after injection, followed by an increase after 24 h.

The bactericidal activity of the macrophages obtained 24 h after treatment of the animals with ASE and LPS separately also was increased (Table 3). In the case of combined administration of these toxins, however, the ingestion function did not show a significant change, but the degree of completion of phagocytosis fell sharply. It must be pointed out that investigation of the morphologic composition of the mouse peritoneal exudate cells 24 h after injection of ASE and LPS showed no significant differences when expressed as percentages of the number of macrophages in the experimental groups of animals. It can therefore be concluded that changes in bactericidal and phagocytic functions revealed by these experiments were due to the effect of the test toxins.

To determine the precise character of the effect of ASE and LPS on macrophagal function, the next series of experiments was carried out in a system in vitro. For this purpose, resident peritoneal macrophages obtained from intact animals were incubated with toxins for 24 h. In this particular case the ingestion function was changed by a lesser degree (Table 4). Bactericidal activity, just as in the experiments in vivo, was enhanced by the action of ASE and LPS separately. After simultaneous addition of the toxins to the macrophages, the bactericidal function was enhanced, but under conditions approaching those in vivo, i.e., when LPS was added 4 h after ASE, the ability of the macrophages to kill Staph. aureus cells also was sharply reduced.

Thus after combined administration of ASE and LPS there was a sharp decline in the function of completed phagocytosis of the macrophages. The fact that in vitro the same patterns were observed as in the system in vivo suggests that SE has a direct action on macrophagal functions. Considering that phagocytic cells constitute the first line of defense against extremely widespread conventionally pathogenic microorganisms, weakening of the bactericidal properties during the synergic action of SE and endotoxins of Gram-negative bacteria in vivo may lead to the development of severe septic complications.

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OBTAINING MONOCLONAL ANTIBODIES TO HUMAN GROWTH HORMONE AND THEIR CHARACTERISTICS

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UDC 615.373.03:[616.154.814.32+616-008.848.143.2]:577.175.322]-078.333].012

KEY WORDS: monoclonal antibodies; hybridoma; human growth hormone; immunoblotting

Growth hormone or somatotrophin (STH) is a pituitary polypeptide hormone with molecular weight of 22 kD. This hormone is characterized by the diversity of its biological action and by its well-marked species-specificity. STH of animal origin has no growth effect in man and virtually does not give any cross reaction with antiserum to human STH.

The solution of certain scientific and practical problems requires quantitative determination of STH in biological fluids and tissue extracts. Until recently, polyclonal antibodies obtained from hyperimmunized animals have been used in diagnostic immunoassay systems. Nowadays, with the development of new and improvement of existing immunochemical methods of determination of human STH, as well as of other protein hormones, monoclonal antibodies (McAb) are beginning to be used on an increasing scale [3, 10], because of the stability of their properties and their high specificity. The use of McAb directed toward particular epitopes in the STH molecule enables reagents to be standardized and makes the process of creation of diagnostic systems technologically more refined.

The aim of this investigation was to obtain hybridomas producing McAb to human pituitary STH (STH_{pit}), and to study the properties of the McAb thus obtained and the possibility of using them in immunoassay systems.

EXPERIMENTAL METHOD

Highly purified preparations of human STH_{pit}, and also human prolactin (PRL), human placental lactogen (PL), bovine STH, bovine PRL, and porcine PRL, isolated in the Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, by methods according to [14, 7, 5, 2], respectively, were used. As well as STH_{pit}, a biosynthetic preparation of human STH obtained by a genetic engineering method (STH_{bio}) in connection with the "Human Growth Hormone" Program of the Academy of Sciences of the USSR, the Ministry of the Medical and Microbiological Industry of the USSR, and the Ministry of Health of the USSR, also was used.

To obtain hybridomas, BALB/c mice were immunized with highly purified STH_{pit}. The course of immunization consisted of three injections of the preparation (100, 50, and 50 μg) in Freund's complete adjuvant with intervals of 4 weeks between injections. Before fusion

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