Leukocytic Response in Monkeys Challenged with Staphylococcal Enterotoxin

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ABSTRACT

SUGIYAMA, H. (University of Chicago, Chicago, Ill.), AND E. M. MCKISSIC, JR. Leukocytic response in monkeys challenged with staphylococcal enterotoxin. *J. Bacteriol.* 92:349-352. 1966—The feeding of staphylococcal enterotoxin to monkeys elicited a leukocytosis which was evident within 0.5 hr of challenge. The peak neutrophilic leukocytosis was reached in 3 hr, and then subsided so that leukocyte counts were normal within 28 hr. Each of the three serological types of enterotoxin tested induced the same effects. Intravenous injection of enterotoxin slightly above the emetic ED_{50} level produced an initial leukopenia followed by a neutrophilic leukocytosis which was maximal 9 or more hr postinjection. With smaller intravenous challenges, some animals responded with a leukopenia followed by a leukocytosis, some with only a leukocytosis, and others with no significant change in total leukocyte counts. The reversal of normal lymphocyte-to-neutrophile ratio toward a neutrophile-predominant white blood cell population occurred in all animals.

Staphylococcal food poisoning results from the action of the enterotoxin elaborated into the food during the growth of some of the coagulase-producing strains of *Staphylococcus* (10). The mode of action of enterotoxin has not been explained, but the similarity in animal responses provoked by the food-poisoning toxin and bacterial endotoxin (lipopolysaccharide, LPS) may be significant. Enterotoxin has been found to elicit animal results comparable to a wide range of effects described for LPS (13). These include the early development of a transitory refractory state to a second emetic challenge (14), sensitization of mice and rabbits to the lethal effect of LPS by prior administration of enterotoxin (18), biphasic febrile response (7), substitution for LPS as the preparative dose of the local Shwartzman reaction (4), sensitization of the skin so that intradermal epinephrine provokes a local hemorrhagic response (4), enhancement of vasoconstrictive action of epinephrine on the peripheral circulatory vessels of the meso-appendix (*unpublished data*), increased responsiveness of monkeys to the emetic stimulus of enterotoxin administered per os after reticulo-endothelial system blockade (17), biphasic effect on the granulopoietic activity of the reticulo-endothelial system (13), hyperfibrinogenemia

(19), thrombocytopenia and related lowered blood serotonin level (19), similarity in sites of emetic action of enterotoxin and LPS (8, 15, 16), hyperlipemia in which the triglyceride fraction shows the greatest proportionate increase with reversal of the normal ratio of esterified to free cholesterol (*unpublished data*), and the ability of an antiprotease (Trasylol) to inhibit the LPS-induced local Shwartzman phenomenon as well as to suppress significantly the emetic action of the food-poisoning toxin (13).

A characteristic response of animals to the intravenous injection of bacterial endotoxin is the change in the numbers of the circulating white blood cells. The classical picture is an immediate leukopenia succeeded by a neutrophilic leukocytosis which attains its peak 6 to 8 hr postinjection (1, 2). The similarities in animal reactions induced by staphylococcal enterotoxin and LPS suggested that the administration of the food-poisoning agent to monkeys would be reflected in a leukocytic response.

MATERIALS AND METHODS

The three serologically different enterotoxins A, B, and C (3, 6) used were essentially pure preparations (95% or more purity). The emetic ED_{50} of these samples for previously unused (new) monkeys by the intragastric (ig) route was approximately 10 μ g per animal and 0.1 to 0.2 μ g/kg by intravenous (iv) injection. Enterotoxins were prepared by M. S. Bergdoll, Food Research Institute. Bacterial endotoxin was *Salmonella*

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enteritidis lipopolysaccharide (Difco, control 131243) solution heated at 85 C for 10 min. Vomiting resulted in one of four monkeys injected iv at a level of 30 µg/kg. Toxins for per os administration were dissolved in 50 ml of water and for iv challenge, in pyrogen-free saline.

The average body weight of the rhesus monkeys (*Macaca mulatta*) was approximately 2.5 kg. Some were new animals without previous experimental experience with enterotoxin; others had one or two previous contacts with enterotoxin of a different serological type from the one being used for the test. Animals resistant to one or more enterotoxin types were used for tests with LPS and as controls (fed water or given iv saline).

Leukocytes were counted by the usual hemacytometer procedure on blood obtained from a leg vein, and differential counting was done on blood films prepared with Wright's stain. A blood sample was obtained immediately before and at selected time intervals after challenge. The 24-hr postchallenge samples actually ranged from 24 to 28 hr.

RESULTS

The leukocyte count for all prechallenge blood samples was $14,400 \pm 2,500/\text{mm}^3$ (mean $\pm$ standard deviation), with $69 \pm 9\%$ lymphocytes and $26 \pm 9\%$ neutrophils. Only monkeys whose leukocyte counts at this time were 10,000 to 20,000/ mm^3 , and who had 50% or more lymphocytes and less than 10% eosinophiles, are included in the results summarized in Table 1.

The feeding of enterotoxin stimulated a leuko-

cytosis whose inception was recognizable within 30 min of challenge. The change toward a neutrophile-predominant population was also evident at this time. The granulocytic peak was reached about 3 hr postchallenge, with a return to normal values by the next morning. Although very immature neutrophils did not appear, 5 to 10% of the total leukocytes at the time of maximal counts were in the "stab" cell category. These cells were rare in normal blood.

The changes occurred independently of the emetic response of the monkeys to enterotoxin. This was confirmed in a different group of four monkeys which had acquired partial immunity to the emetic action of enterotoxin C from prior feedings of the homologous enterotoxin. When fed 20 µg of the C toxin, none of the animals vomited, but 3 hr after enterotoxin feeding the average prechallenge white-cell count of 14,200/ mm^3 with a 66% lymphocyte to 30% neutrophile ratio had increased to 26,500/ mm^3 with 80% neutrophils.

The leukocytic responses after enterotoxin given by the iv route varied with the challenge levels. Enterotoxin B and C doses of 1 to 1.5 µg/kg stimulated a biphasic change in which a leukopenia preceded the development of the granulocytosis (Table 1). Maximal leukocytosis was attained later than that which followed per os administration of enterotoxin, although a more severe peak granulocytosis occurred. Neutro-

TABLE 1. *Effects of staphylococcal enterotoxin and bacterial endotoxin on numbers of circulating white blood cells of monkeys^a*

Challenge			No. vomiting/ no. tested	Pre- challenge	Postchallenge						
Type	Route	Amt (µg) ^b			0.5 hr	1 hr	3 hr	5 hr	7 hr	9 hr	24 hr
Control			0/8	14,200 (74:25)	—	16,000 (76:20)	15,600 (71:27)	14,700 (70:27)	13,500 (68:24)	—	14,300 (72:25)
A	ig	10	1/5	13,600 (70:25)	17,500 (44:55)	22,100 (32:67)	27,100 (23:72)	23,300 (25:74)	20,900 (27:71)	16,900 (44:53)	12,400 (63:42)
B	ig	10	2/5	14,400 (69:26)	16,400 (57:38)	21,500 (37:61)	36,400 (18:81)	28,400 (26:73)	25,800 (29:63)	—	14,100 (75:22)
C	ig	5	3/4	13,500 (70:25)	19,000 (40:59)	22,100 (35:62)	29,200 (25:73)	23,200 (27:71)	21,600 (37:63)	20,200 (51:44)	13,100 (66:31)
B	iv	1	2/4	13,700 (66:27)	9,000 (49:47)	10,200 (40:57)	13,300 (20:79)	14,300 (10:89)	18,900 (8:90)	22,500 (7:91)	11,000 (43:51)
C	iv	1-1.5	3/4	15,000 (70:26)	11,700 (45:52)	8,700 (50:47)	10,800 (33:65)	15,100 (17:81)	17,900 (16:81)	23,000 (9:89)	13,000 (41:54)
Endo- toxin	iv	5	0/6	15,800 (61:35)	10,700 (57:42)	10,300 (51:46)	20,300 (18:81)	29,100 (20:78)	27,900 (20:78)	20,200 (41:58)	17,200 (63:32)

^a Results are expressed as average total count per cubic millimeter in blood of animals in each experimental group. Figures in parentheses are average lymphocyte-neutrophile percentages in differential counts.

^b Intragastric (ig) doses are given as amount per animal; intravenous (iv) doses, as amount per kilogram of body weight.

philic stab cells ranged up to 20% of the total leukocyte count.

A biphasic effect was induced with the dosage of iv LPS used. The results for control monkeys given water per os or saline iv are combined in the table because they were similar.

The results were more variable with smaller iv challenges of 0.1 to 0.5 $\mu\text{g/kg}$ of enterotoxin B or C. Of eight monkeys injected with these doses, two showed no significant change in the total leukocyte count and three had the biphasic response. The remaining five reacted with an immediately developing neutrophilic leukocytosis such as that which followed the feeding of enterotoxin, but the leukocytosis peaks were reached within 1 to 7 hr of toxin injection. All the monkeys showed a change to a relative neutrophilic granulocytosis in which the neutrophils comprised more than 65% of the total leukocytes counted. No correlation between the occurrence of emesis and the type of leukocytic response was apparent.

These observations indicate that the dosage levels of iv enterotoxin used to obtain the tabulated data were at or near the critical level necessary to elicit the biphasic leukocytic reaction. For this reason, the leukocyte results with one monkey injected with enterotoxin B and another injected with enterotoxin C (1 $\mu\text{g/kg}$) have not been included in the table. These animals developed an immediate granulocytosis and presumably were slightly less sensitive to the action of enterotoxin, which stimulates the biphasic reaction.

DISCUSSION

The incubation period in human staphylococcal food poisoning is a few hours; similarly, the interval to the initial vomiting episode of monkeys fed enterotoxin is usually 1.5 to 3.5 hr. Nevertheless, the observable indication of leukocytosis within 30 min of enterotoxin feeding is surprising. The present results, obtained under experimental conditions corresponding to the natural means of entry of enterotoxin into animals in food poisoning, suggest an almost immediate toxic reaction upon entrance of enterotoxin into the gastrointestinal tract.

The similarity in the changes in the circulating white blood cell populations of monkeys after iv injections of staphylococcal enterotoxin and bacterial endotoxin was not unsuspected (13). Both categories of toxin can elicit a biphasic change in the leukocyte counts with proper dosage. That the higher iv challenge doses of enterotoxin are needed to provoke the biphasic response is supported by data of a recent paper in which a massive iv dose (100 $\mu\text{g/kg}$) of enterotoxin B, of

purity comparable to that used in the present work, seems to have given consistent biphasic leukocytic responses (9). The induction of the initial leukopenia by LPS has also been related to dosage (1, 11, 12).

There is evidence that iv-administered enterotoxin (9) and LPS (5) may be rapidly bound to leukocytes, and that these white cells are then sequestered in organs such as the lungs. Such a mechanism would explain the leukopenia. On this basis, the absence of leukopenia after the feeding of enterotoxin suggests that enterotoxin does not enter into the blood stream in an amount sufficient to affect the leukocytes so that they would be removed from the general circulation. However, the ability of enterotoxin administered per os to mobilize the granulocytes from their depots is incontestable.

One interpretation of the data presented would be that enterotoxin, in relative terms of doses needed to provoke the same emetic effect, is less effective in inducing leukocytosis when administered iv than ig. However, this is probably only an apparent result. The smaller iv challenge may actually call forth leukocytes into the general circulation, but this response may be masked by the initial leukopenia; a detectable leukocytosis does not occur until the leukopenic effect subsides or becomes small relative to the leukocytosis response. The evidence of a rapid shift in the lymphocyte-neutrophile ratio and the late maximal leukocytosis obtained in the biphasic response to iv enterotoxin support this conclusion. Moreover, the two monkeys which did not give a significant increase in total leukocyte counts after the small doses of enterotoxin developed a relative neutrophilic granulocytosis.

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