Inhibition by Endotoxin of the Migration of Peritoneal Exudate Cells from Endotoxin Sensitive Mice (33076)

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Recent studies (1–3) have provided experimental evidence strongly supporting the hypothesis (4) that an acquired hypersensitivity of the delayed type plays an important role in determining host reactivity to the bacterial endotoxins. The inhibition of migration of specifically sensitized peritoneal exudate cells in the presence of antigen, as an *in vitro* model of delayed hypersensitivity has shown good correlation with delayed skin reactivity for tuberculin, diphtheria toxoid, ovalbumin, and dinitrophenylated protein (5–7). The present report describes the application of the cell migration method to our studies on the mechanisms determining host reactivity to endotoxins (8).

Materials and Methods. Female CD-1 mice (Charles River) weighing 18–20 gm were used. Animals were allowed access to food and water *ad libitum*. Boivin endotoxins (Difco) of the following organisms were used: *Salmonella abortus-equi, Serratia marcescens,* and *Escherichia coli* 0127:B8. Experimental animals received 0.01 μg of the various endotoxins administered as a single i.p. injection in nonpyrogenic saline, while control animals received saline. Exudates were produced in experimental and control animals by the i.p. injection of 1.0 ml of sterile mineral oil (Marcol 52, Esso) 3 days prior to the harvest of cells for migration studies. Mice were sacrificed by cervical dislocation. Immediately, 10 ml of Eagle's minimal essential medium (Grand Island Biological Company) diluted 1–4 with saline was injected i.p. to suspend the peritoneal exudate cells. Abdominal skin was reflected and the animal was positioned on an open wire rack so that the peritoneal exudate could be drained by a 20-gauge needle into a centrifuge tube placed below the animal. Exudates thus collected were for the most part free of erythrocytes. Separately collected exudates were centrifuged at 4°C, 1000 rpm for 5 min, and mineral oil and supernatant fluid was poured off. Cells were washed twice by centrifugation and resuspension in 5-ml volumes of undiluted Eagle's medium. The cell suspension was drawn into glass melting point tubes (Scientific Glass Apparatus Co.), 1 × 150 mm, and the tube sealed at one end with heat from a microburner. Centrifugation yielded a column of packed cells 3–4 mm long. Tubes were broken at the cell–liquid interface after scribing with a diamond pencil. The portion of the tube containing the cells was pasted with silicone grease to the bottom of a glass dish, 20 mm o.d. × 4 mm deep. Four such tubes were routinely placed in each dish. The dish was filled with medium premixed to contain 10% fresh normal mouse serum and the desired concentration of the various endotoxins. A coverslip sealed the dish. After incubation at 37°C for 24 hours, cells were seen to have migrated out of the capillary tube onto the bottom of the dish forming fan-shaped areas. The image of the area occupied by the migrating cells was projected onto paper and drawn. The absolute area was determined by planimetry with reference to a projected standard square millimeter.

Results. From the data given in Table I it can be seen that equivalent maximum migration was achieved in medium not containing endotoxin by cells of normal animals and by those cells taken from animals 5, 10, and 20 days after endotoxin injection. Cells of normal mice and cells taken 5, 10, and 20 days after endotoxin injection migrated poorly in the presence of 1.0 μg/ml of the various endotoxins. A higher concentration of endotoxin in the medium (10 μg/ml) did not effect a greater inhibition than that noted at 1.0 μg/ml. Medium containing 0.01 μg of the various endotoxins was not inhibitory to cells of normal mice. In contrast, cells harvested from mice 10 days after treatment with *S. abortus-equi* or *E. coli* endotoxins migrated

TABLE I. Influence of Endotoxins on the Migration of Peritoneal Exudate Cells Harvested 5, 10, or 20 Days after Treatment of Mice with the Respective Endotoxin (0.01 μg).

Cell donor treatment[a]	Days after donor treatment	Cell migration (mm^2)[b] Endotoxin concentration (μg/ml) 0.0 A	0.0 B	0.01 A	0.01 B	1.0 A	1.0 B	10
None	—	4.3	5.7	4.5	5.0	0.9	0.6	0.5
E.c.	5	5.1	5.5	3.9	5.9	2.3	0.7	0.7
None	—	5.4	6.1	4.3	5.3	2.3	1.1	0.9
E.c.	10	5.1	5.7	0.8[c]	1.6[c]	0.5	1.0	0.7
None	—	5.1	5.4	4.1	5.7	1.2	1.4	0.9
E.c.	20	4.6	4.9	4.3	6.1	1.5	1.1	1.6
None	—	4.3	5.7	4.7	5.3	1.5	0.9	0.8
S.a.e.	5	4.9	5.2	5.3	4.8	1.4	0.8	0.6
None	—	5.4	6.1	5.3	6.1	2.4	0.5	1.8
S.a.e.	10	5.1	5.7	1.6[c]	0.8[c]	2.3	0.9	0.8
None	—	5.1	5.4	3.5	5.9	1.4	2.0	1.0
S.a.e.	20	5.2	5.1	4.2	5.7	1.4	0.7	1.2
None	—	4.3	5.7	5.3	5.0	2.4	0.7	0.6
S.m.	5	4.7	5.9	4.1	5.5	0.8	0.8	1.3
None	—	5.4	6.1	4.4	5.7	1.9	1.0	0.5
S.m.	10	5.8	5.9	5.2	5.4	2.6	0.8	1.7
None	—	5.1	5.4	3.9	5.4	1.6	0.7	0.9
S.m.	20	5.2	5.4	3.2	6.4	1.7	1.1	1.4

[a] Endotoxins: E.c., *E. coli*; S.a.e., *S. abortus-equi*; S.m., *S. marcescens*.

[b] Cells harvested from 4 mice/group for each of the two experiments (A and B). Values are means of 4 determinations.

[c] Values significantly differ from respective control values at $p < 0.001$. Values for 1.0 and 10 μg/ml differ significantly from controls.

poorly in the presence of 0.01 μg/ml of the respective endotoxins. This susceptibility to inhibition of migration at 0.01 μg/ml was not found for cells harvested at 5 or 20 days after endotoxin treatment of the cell donors. For the *S. marcescens* endotoxin, treatment of the cell donors did not influence the behavior of the cells harvested at 5, 10, or 20 days, although this endotoxin was as effective as the others in modifying the migration of cells harvested from normal mice.

Discussion. Our recent studies on modification of host reactivity to subsequent endotoxin challenge (1–3,8) have dealt with induced hyperreactivity revealed by delayed skin reactivity in rabbits and by susceptibility of mice to toxic and immunologic influences of endotoxins. The hypersensitivity induced by a variety of endotoxins was transferable by spleen cells but not by serum (1,3), required the protein component of the sensitizing endotoxin (1,2), and for the ordinarily inactive *Brucella* preparation required administration with complete Freund's adjuvant (3). The present data establish that exudate cells harvested from mice at that time when the cell donors have been shown to be hypersensitive to endotoxin behave *in vitro* in the presence of the endotoxin, in terms of susceptibility to inhibition of migration, as do specifically sensitized cells in the presence of antigen in classical delayed hypersensitivity states (5–7).

That normal host reactivity to endotoxins derived from those gram-negative bacteria to which animals are commonly exposed may have an origin in a naturally acquired hypersensitivity has been emphasized (4,8,9) and the influence of the higher concentrations of endotoxin on "normal" cells may in part be an expression of this preexisting sensitivity. The susceptibility of normal cells to common

enterobacterial endotoxins has been amply demonstrated (10–15). That a separate primary cytotoxicity may be operative in the case of endotoxins, analogous to the separate primary toxicity postulated for *in vivo* endotoxic responses (9) must be considered. The susceptibility of normal macrophages to common endotoxins did not extend to a *Brucella* endotoxin (14) a finding emphasizing the importance of previous immunological experience and with which our findings (3, 8) distinguishing reactivity to enterobacterial and *Brucella* endotoxins are entirely consistent. The failure of the *S. marcescens* endotoxin to modify reactivity of subsequently harvested cells, despite an activity for normal cells equivalent to the *S. abortus-equi* and *E. coli* endotoxins, is also relevant. For the time interval reported, the same *S. marcescens* preparation failed to elicit a hyperreactive response in endotoxin sensitized mice despite its typical activiy in normal mice (1) consistent with the present *in vitro* findings. Subsequent work (16) has established that the S. *marcescens* endotoxin induces a relatively long-lasting immunity ("tolerance") (9) in mice and rabbits and that the appearance of hypersensitivity is much delayed in comparison with the other endotoxins. Further studies on host reactivity to commonly and uncommonly experienced endotoxins and on the role of the protein components in sensitization, taking advantage of the opportunity afforded by the *in vitro* cell migration model, are in progress.

Summary. The influence of various bacterial endotoxins on the migration of mouse peritoneal exudate cells has been examined. Pretreatment of cell donors with *S. abortus-equi* or *E. coli* endotoxins rendered cells, harvested 10 days later, more susceptible to inhibition by the respective endotoxins. This increased reactivity was not in evidence for cells harvested 5 or 20 days after treatment. Cells from *S. marcescens* endotoxin treated animals did not exhibit a change in their susceptibility to that endotoxin during the 20-day test period. These findings are entirely consistent with previous *in vivo* studies supporting the hypothesis that host reactivity to endotoxins has an origin in an acquired hypersensitivity of the delayed type.

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Received Feb. 5, 1968. P.S.E.B.M., 1968, Vol. 128.