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Species Differences in Kupffer Cells and Endotoxin Sensitivity

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The relative species sensitivity to *Escherichia coli* O111:B4 endotoxin was found to be guinea pig > hamster > mouse > rat. The 50% lethal dose of this endotoxin correlated with both the rate at which single latex particles were phagocytosed by individual Kupffer cells and the number of Kupffer cells in hepatic lobules that phagocytosed latex. The results suggest that the intrahepatic density and the level of activation of Kupffer cells participate in determining endotoxin sensitivity.

Kupffer cells (KCs) are one of the cellular components of the lining of sinusoids in the liver and are the principal cells involved in the removal of circulating endotoxins (ETs) from the blood (9, 10, 19). After endocytosis of an ET, a variety of beneficial and toxic mediators are released from the KCs; these are thought to participate in the host response to this toxin (1, 2, 4–8, 11, 17, 18). Since different species have widely different sensitivities to ET (20, 21, 23), this study investigated whether any correlation existed between sensitivity to ET and the number, distribution, and phagocytic activity of KCs in the livers of guinea pigs (Hartley), hamsters (Syrian golden), mice (NMRI), and rats (Wistar).

The relative sensitivities of these species to ET were determined concurrently with the same preparation of *Escherichia coli* O111:B4 ET, which was prepared in our laboratory (Mannheim) by the method of Boivin et al. (3). This preparation has a very reproducible, high toxicity level (20, 21). For each species, three groups of animals were established, with six animals in each group. Each group received intravenous injections of one of three different doses from a single batch of ET on the same day to establish the 50% lethal dose (LD_{50}) for each species under similar conditions. The doses used were based on considerable experience with administering similar ET preparations to these species, although not concurrently (20, 21), and were chosen to bracket the LD_{50}. To facilitate comparisons between species of very different body weights (guinea pigs, 225 to 275 g; hamsters, 90 to 110 g; mice, 25 to 30 g; rats, 210 to 235 g), the doses were as follows; guinea pigs, 0.75, 1.0, and 1.25 μg/g of body weight; hamsters, 2, 3, and 4 μg/g; mice, 2, 4, and 8 μg/g; and rats, 4, 6, and 8 μg/g. The LD_{50} was calculated from a graph of the percentage of animals which died over a 6-day period versus the dose of ET.

Subsequently, the livers from six additional animals of each species were studied by high-resolution, in vivo microscopy methods (12–16) while the animals were anesthetized with urethane (0.03 mg/g of body weight). The number and distribution of KCs that had phagocytosed latex particles were determined 15 min after a standardized intraportal infusion of 1.0-μm latex particles (10^4 particles per g of body weight) suspended in 0.1 ml of pyrogen-free saline. The number of phagocytic KCs per standardized microscopic field (9,415 μm^2) was then counted for each liver in 10 periportal and 10 centrilobular fields. This concentration of latex particles was optimal for identifying all phagocytic KCs, since subsequent injections increased the number of phagocytosed latex particles per KC without changing the number of identifiable KCs. Similar numbers and distributions of phagocytic KCs were also observed when the latex was injected intravenously before anesthesia.

In another set of anesthetized animals, consisting of six animals of each species, the rate at which latex particles were phagocytosed by 10 different KCs in each liver was determined by measuring the time required for individual KCs to internalize single latex particles once they touched the cell surface (12, 14–16).

The mean values of the data for each animal were determined, and the data for each species were expressed as the mean ± the standard error of the mean for each group. The Student t test was used to determine significant differences between groups; $P < 0.05$ was considered significant. Regression analysis was used to determine correlations between the various groups; $r > 0.95$ was considered significant.

The toxicity studies confirmed previous findings (20, 21, 23) that, of the species tested, the guinea pigs were the most sensitive to ET; the 6-day LD_{50} of *E. coli* O111:B4 ET was 0.75, 2.0, 5.0, and 7.0 μg/g of body weight for guinea pigs, hamsters, mice, and rats, respectively. However, in contrast to the guinea pigs, mice, and rats that died, almost all of which did so within 24 h, the hamsters responded slowly and none died until 3 days after the injection of ET (Table 1).

The number of periportal and centrilobular KCs that phagocytosed the latex particles was similar within a species but differed significantly ($P < 0.01$) between guinea pigs, mice, and rats (Table 2). Although the number in hamsters differed significantly ($P < 0.01$) from that in guinea pigs and rats, the difference in number between the hamsters and the mice was not quite statistically significant ($0.05 < P < 0.1$). The overall numbers of phagocytic KCs were therefore guinea pig > hamster ≥ mouse > rat. Nevertheless, linear regression analysis showed significant correlations between the LD_{50} for all species and the number of KCs in both the periportal ($r = 0.98$) and the centrilobular ($r = 0.95$) regions of the hepatic lobules.

In each species, there was a periportal-to-centrilobular gradient in the KC density, with the periportal regions containing the largest number of KCs that phagocytosed latex

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TABLE 1. Time to death for animals administered *E. coli* O111:B4 ET[a]

Species[b]	No. dead on day:					
	1	2	3	4	5	6
Guinea pig	13	0	0	0	0	0
Hamster	0	0	2	6	3	1
Mouse	8	1	0	0	0	0
Rat	7	1	0	0	0	0

[a] Within the range of doses used to establish an LD_{50}, the time of death was dose-independent.
[b] There were 18 animals of each species.

particles (Table 2). Although this gradient differed between the four species, with the guinea pigs and rats having the smallest and largest gradient, respectively, the gradient did not correlate with the LD_{50} for any of the species.

The rate of phagocytosis of the latex particles by the KCs showed little variation within a given species but differed significantly ($P < 0.01$) between species. The mean time (± standard error of the mean; $n = 6$) required for individual KCs to phagocytose single particles was 18.4 ± 1.6, 23.6 ± 1.7, 27.2 ± 1.2, and 31.4 ± 1.7 s for guinea pigs, hamsters, mice, and rats, respectively. There was a significant correlation between the rate of phagocytosis and the LD_{50} of ET for these species ($r = 0.97$).

The results of this study showed that a strong correlation existed between sensitivity to ET (LD_{50}) and the number and phagocytic activity of the KCs in guinea pigs, hamsters, mice, and rats. This is consistent with the KCs being the principal site for the removal of circulating ET (9, 10, 19) and the source of various beneficial and toxic mediators that participate in the host response to this toxin (1, 2, 4–8, 11, 17, 18). Although the rate of phagocytosis reflects the functional state of the KCs, it may not reflect endocytotic activity involved in ET clearance. However, the relationship between phagocytic activity and the release of toxic mediators from these cells is widely recognized. In both respects the results are consistent with those of our previous studies, which showed low numbers of phagocytic KCs in the livers of C3H/HeJ mice with little sensitivity to ET, and increased phagocytic activity in mice highly sensitive to ET whose KCs had been activated with *Mycobacterium bovis* BCG (14–16).

The difference in KCs density between hamsters and mice was not statistically significant at the 95% confidence level; this may be related to the considerably longer time to death (3 to 6 days) for the hamsters. Although the reasons for this delay are not yet known, they may include differences in mediator production and release, as well as humoral and cellular immunological factors (24). Nevertheless, most of the evidence suggests that the intrahepatic density of KCs and their level of activation affect the sensitivity of the host to circulating ET.

TABLE 2. Number of KCs which phagocytosed latex particles

Species	No. (mean ± SEM)[a] of phagocytosing KCs per:		
	Periportal field	Centrilobular field	Mean
Guinea pig	12.0 ± 0.6	7.6 ± 0.5	9.9 ± 0.4
Hamster[b]	10.2 ± 0.5	4.2 ± 0.5	7.2 ± 0.5
Mouse[b]	8.9 ± 0.8	5.4 ± 0.7	7.1 ± 0.7
Rat	5.9 ± 0.4	2.0 ± 0.6	4.0 ± 0.3

[a] For six animals of each species, the number of KCs in 10 periportal and 10 centrilobular fields in each liver was counted.
[b] Not statistically different from each other ($0.05 < P < 0.1$).

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