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AGING AND THE RESPONSE TO SALMONELLA INFECTION

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Abstract — We investigated the effect of age on the ability of Fischer 344 rats to mount a febrile response and contain infection due to *Salmonella typhimurium*. Elderly (22–23 month), middle-aged (12–13 month) and young (2–3 month) rats were inoculated intraperitoneally with 1.5×10^6 organisms and the febrile response and liver and spleen bacterial counts were followed for 13 days. The elderly had a more sluggish febrile response and did not achieve as great a maximum temperature elevation as the young and middle-aged rats. Except for days 1 and 5, bacterial counts in liver and spleen were greater in the elderly rats than in young and middle-aged rats.

Key Words: Salmonella infection, aging, fever, aged rats, host defense

INTRODUCTION

ELDERLY HUMANS have increased mortality from many different types of infections (Gardner, 1980). The reasons for the increased mortality have not been entirely elucidated, but most likely, multiple factors are involved. One of these factors could be a decreased febrile response, which has been correlated with increased mortality (Kreger *et al.*, 1980; Weinstein *et al.*, 1983).

The association between diminished fever and increased mortality seems to be especially true in elderly individuals (Finkelstein *et al.*, 1983). Although the course of infection with several pathogens has been studied in aged animals (Gardner and Remington, 1977; Emmerling *et al.*, 1979; Patel, 1981a,b; Louria *et al.*, 1982), there is only one prior study of the febrile response during infection in aged animals (Tocco-Bradley *et al.*, 1985). In this study, we explored the effects of age on the febrile response and on the ability to handle infection using as a model Salmonella infection in Fischer 344 rats.

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MATERIALS AND METHODS

Animals

Specific pathogen-free young (2–3 month), middle-aged (12–13 month) and elderly (22–23 month) Fischer 344 male rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) were housed in individual wire mesh cages and maintained on a 12-h light–dark photoperiod at an ambient temperature of 26 ± 1°C. Tap water and rodent chow were provided *ad libitum*.

Determination of body temperature

The week prior to the experiment, battery operated biotelemetry devices (Mini-Mitter, Inc., Sun River, OR) were implanted intraperitoneally (i.p.) into rats to be used for febrile response studies. Output from each transmitter, monitored by an AM radio receiver, was proportional to temperature, as described previously (Bradley *et al.*, 1987; Tocco-Bradley *et al.*, 1985).

Salmonella typhimurium infection

S. typhimurium, ATCC 15277, stored in glycerol at −20 °C was grown overnight in brain heart infusion (BHI) broth, diluted in 0.9% NaCl to 1–2 × 10^6 CFU/ml, and injected i.p. in a volume of 1 ml. The actual CFU/ml used for inoculation was verified by pour-plate techniques using BHI agar. Within each age group, seven rats were used for febrile response studies and 84 rats were used for assessment of liver and spleen bacterial colony counts.

Febrile response assay

The seven rats in each age group used for these studies had been implanted with biotelemetry devices one week previously. Baseline temperatures were measured every 6 h for 72 h prior to injection of *S. typhimurium* and averaged for each age group for both light (4 a.m. to 4 p.m.) and dark (4 p.m. to 4 a.m.) cycles. Body temperature was measured every 6 h for 13 days after injection of *S. typhimurium* and expressed as the change in temperature from baseline.

Determination of liver and spleen bacterial colony counts

Every other day after injection of *S. typhimurium* (beginning with the day after injection as day 1), 12 rats in each age group were sacrificed by cardiac exsanguination following methoxyflurane anesthesia. A portion of the liver and the entire spleen were aseptically removed, weighed, homogenized in sterile water to lyse the cells and release bacteria, serially diluted in 0.9% saline and added in duplicate to BHI agar using standard pour-plate techniques. After overnight incubation at 37 °C, the number of colonies were counted, the counts on the two plates were averaged, and the results were expressed as CFU/g tissue.

Data analysis

Febrile response data were analyzed using Student's *t* test with the Bonferroni correction for multiple groups. Differences in liver and spleen bacterial loads were analyzed by the Kruskal–Wallis test for nonparametric comparisons of groups.

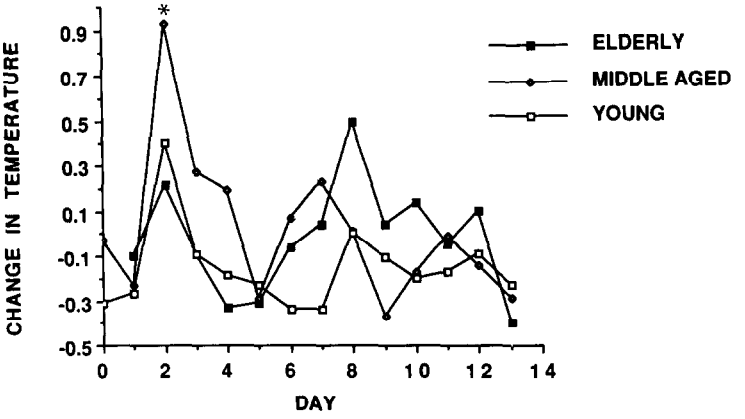

FIG. 1. Light cycle changes in temperature from baseline of young, middle-aged and elderly rats, in whom temperatures were measured every 6 h for 13 days following an intraperitoneal injection of 1.5 $\times$ 10^6 CFU *S. typhimurium*. Each point represents the mean daily light cycle temperature for seven rats. *$p < 0.05$.

RESULTS

In this experiment, as well as prior experiments, we found that temperatures within each light or dark cycle remained relatively constant; therefore, for each day, we averaged the values within each light and each dark cycle to give a mean daily light and dark cycle temperature.

The febrile response to Salmonella infection was most clearly noted during the light cycle when the rats normally are less active and have their lowest body temperatures. The major

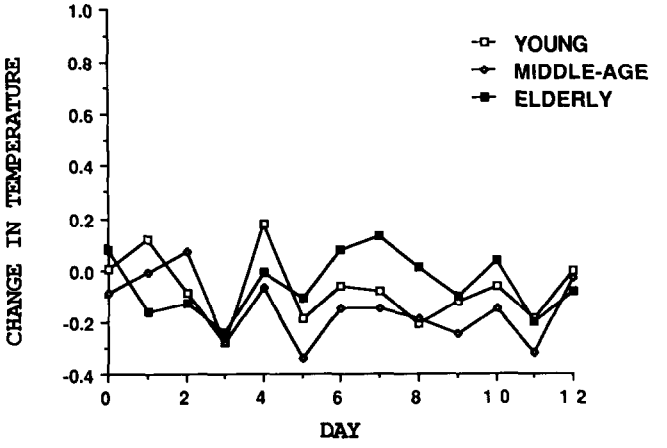

FIG. 2. Dark cycle changes in temperature from baseline of young, middle-aged and elderly rats, in whom temperatures were measured every 6 h for 13 days following an intraperitoneal injection of 1.5 $\times$ 10^6 CFU *S. typhimurium*. Each point represents the mean daily dark cycle temperature for seven rats. No significant differences were noted.

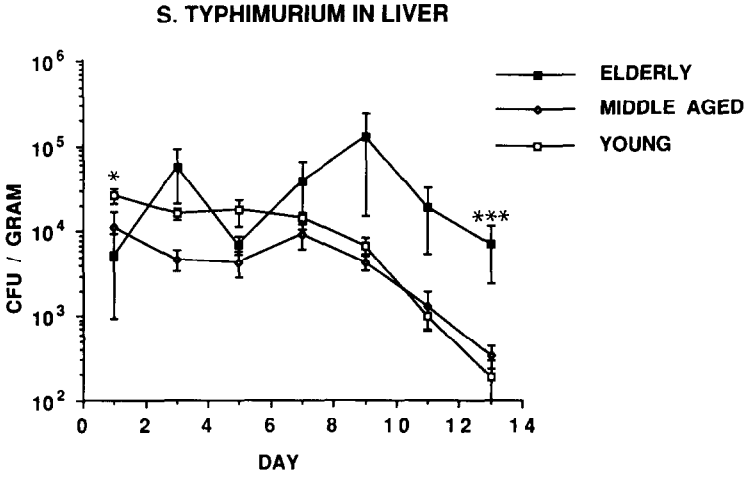

FIG. 3. The number of bacteria per gram of liver over 13 days in young, middle-aged and elderly rats that received 1.5×10^6 CFU *S. typhimurium* intraperitoneally. Each point represents the mean ± SEM for 12 rats. $*p = 0.0045$ for elderly vs. young rats on day 1, $***p = 0.006$ for elderly vs. young rats on day 13.

differences were noted on day 2 when all age groups experienced a change in their circadian temperature cycle, showing a febrile response during their light cycle (Fig. 1). The dark cycle temperatures did not vary between age groups (Fig. 2).

The middle-aged rats had a greater febrile response on day 2 (0.93 °C) than either the aged (0.21 °C) or the young (0.40 °C) rats ($p < 0.05$). The maximum temperature achieved by the middle-aged and young rats was on day 2, while the aged appeared to have a more sluggish response with their maximum temperature achieved only by day 8.

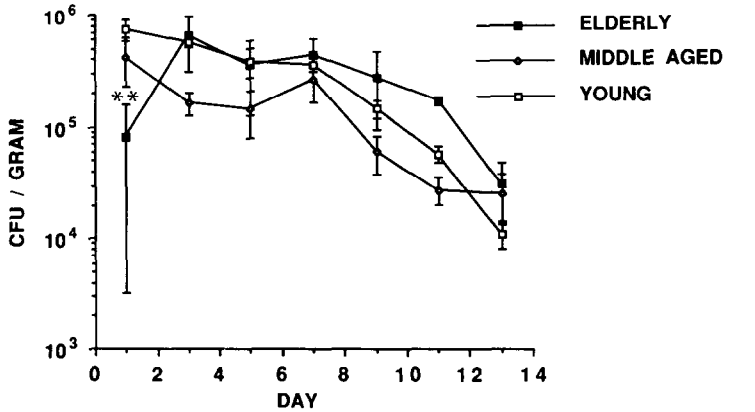

FIG. 4. The number of bacteria per gram of spleen over 13 days in young, middle-aged and elderly rats that received 1.5×10^6 CFU *S. typhimurium* intraperitoneally. Each point represents the mean ± SEM for 12 rats. $**p = 0.001$ for elderly vs. young rats on day 1.

The three groups showed differences in their ability to clear *Salmonella* from the liver and spleen throughout the course of the infection, although the differences between age groups in the spleen bacterial counts were not as great as those in the liver (Fig. 3 and 4). Aged rats had greater numbers of organisms in the liver and spleen than did young and middle-aged rats, except on days 1 and 5. Initially, the aged rats cleared *Salmonella* more efficiently from both liver and spleen, having significantly fewer organisms than the other groups; however, this was followed by a large increase in organisms begnning on day 3. In all three age groups, the spleen contained at least a log more organisms than the liver on each of the days studied.

DISCUSSION

Elderly persons experience an increased number of bacterial infections and manifest higher mortality from these infections (Kreger *et al.*, 1980; Finkelstein *et al.*, 1983; Weinstein *et al.*, 1983). Many physicians have suspected that the major reason for increased mortality from bacterial infections is related to defects in host defense mechanisms in the elderly (Gardner, 1980). Defects in T cell function have been described in elderly humans and experimental animals (Weksler and Hutteroth, 1974; Makinodan and Kay, 1980; Patel, 1981a). Indeed, defects in T cell function have been cited as the possible cause of the inability of aged mice to handle certain pathogens (Gardner and Remington, 1977; Emmerling *et al.*, 1979; Patel, 1981a,b; Louria *et al.*, 1982). Patel showed that antimicrobial defenses against *Listeria monocytogenes* were greater in middle-aged mice than in young and aged mice (Patel, 1981a), a finding similar to ours in Fischer rats with Salmonella infection. Using only two age groups, Emmerling *et al.* (1979) noted that young mice were better able to contain an intravenous challenge of *S. typhimurium* than aged mice. Defects in T cell function might explain the decreased resistance to *S. typhimurium* in Fischer rats, as well, especially since the major differences were noted from day 9 to day 13, when T cell activation of macrophages probably is occurring (Mackaness, 1971).

It is of interest than the elderly rats had significantly fewer organisms in liver and spleen on day 1 than both middle-aged and young rats. It is possible that the elderly were able to mobilize more neutrophils into the peritoneal cavity in response to the Salmonella challenge, as noted previously in elderly mice given *Staphylococcus aureus* and *Streptococcus pneumoniae* challenges (Esposito and Pennington, 1983; Louria *et al.*, 1986).

It is tempting to speculate that the decreased resistance to *Salmonella* may have been related to the diminished febrile response seen in the aged rats. We noted that aged rats showed less fever on day 2, when all three age groups manifested fever in response to the infection, and that aged rats did not achieve their maximum temperature until day 8. However, young rats also showed a diminished febrile response on day 2, yet handled Salmonella infection as well as the middle-aged rats. We do know from prior experiments that aged rats produce less of several different monokines (interleukin-1 and tumor necrosis factor) that are important in both fever generation and host defense (Bradley *et al.*, 1989). No decrease in febrile mediators has been noted in young rats; it is possible that the lower fever in this group relates more to thermoregulatory effector mechanisms and less to production of monokines which are important in host defense.

The current experiments point out a correlation between fever and response to infection but do not establish a causal link between the two. Studies with poikilotherms have established a relationship between fever and resistance to an infecting organism (Vaughn *et al.*, 1974); however, studies in homeotherms, such as rats and humans, are more complex and have not

proved that fever is definitely beneficial to the host. A model of sublethal infection, as used in these experiments, is relevant to the clinical experience with many infections in the elderly and should be explored further in regard to the possible beneficial effects of fever and other components of the acute phase response.

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