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Febrile-Range Hyperthermia Augments Pulmonary Neutrophil Recruitment and Amplifies Pulmonary Oxygen Toxicity

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Febrile-range hyperthermia (FRH) improves survival in experimental infections by accelerating pathogen clearance, but may also increase collateral tissue injury. We hypothesized that FRH would worsen the outcome of inflammation stimulated by a non-replicating agonist and tested this hypothesis in a murine model of pulmonary oxygen toxicity. Using a conscious, temperature-controlled mouse model, we showed that maintaining a core temperature at FRH (39°C to 40°C) rather than at euthermic levels (36.5°C to 37°C) during hyperoxia exposure accelerated lethal pulmonary vascular endothelial injury, reduced the inspired oxygen threshold for lethality, induced expression of granulocyte-colony stimulating factor, and expanded the circulating neutrophil pool. In these same mice, FRH augmented pulmonary expression of the ELR$^+$ CXC chemokines, KC and LPS-induced CXC chemokine, enhanced recruitment of neutrophils, and changed the histological pattern of lung injury to a neutrophilic interstitial pneumonitis. Immunoblockade of CXC receptor-2 abrogated neutrophil recruitment, reduced pulmonary vascular injury, and delayed death. These combined data demonstrate that FRH may enlist distinct mediators and effector cells to profoundly shift the host response to a defined injurious stimulus, in part by augmenting delivery of neutrophils to sites of inflammation, such as may occur in infections. In certain conditions, such as in the hyperoxic lung, this process may be deleterious. *(Am J Pathol 2003, 162:2005–2017)*

Fever is a phylogenetically ancient, evolutionarily conserved response, the key feature of which is a temporary, regulated increase in core temperature. While mammals and birds generate fever through both heat-seeking behavior and augmented heat generation and retention, ectothermic animals increase their body temperature by seeking an external heat source. In both cases, stricken animals expend substantial amounts of energy to achieve an increase in body temperature,[1] suggesting that the increase in temperature itself is beneficial during infections and injury. This conclusion is supported by retrospective clinical studies showing an association between fever and improved survival during bacterial infections[2] and the beneficial effects of febrile range hyperthermia (FRH) in experimental infections in animals. We previously reported that exposing mice to FRH during experimental *Klebsiella pneumoniae* peritonitis increased survival rate from 0% to 50% and decreased the intraperitoneal bacterial load by 100,000-fold compared with euthermic mice.[3] However, FRH did not directly influence pathogen proliferation or survival,[3] suggesting that the salutary effects in the infected host were the result of augmented immune defense.

In the retrospective studies of human infections, the association of fever with survival was lost as the acuity of the patient population increased.[4] Furthermore, in patients with *Escherichia coli* and *Pseudomonas aeroginosa* sepsis, administration of the antipyretic acetaminophen was associated with increased survival.[5,6] Collectively, these studies suggest that fever might be harmful when it occurs in certain clinical settings. In our experimental murine *Klebsiella* peritonitis model,[3] bacterial burden at time of death was much lower in the mice exposed to FRH than the euthermic mice, suggesting that mechanisms other than the infection contributed to death in the warmer mice. A similar effect of FRH on the relationship between survival and pathogen burden occurs in rabbits with experimental *S. pneumoniae* peritonitis in which FRH (41°C) shortens survival time despite reducing levels of

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bacteremia compared with infected euthermic (39°C) animals.[7] Based on these studies, we reasoned that the ultimate effect of fever is determined by a dynamic balance between accelerated pathogen clearance and augmented collateral host tissue injury. Specifically, fever will be beneficial if it sufficiently shortens the course of an infection, thereby reducing the risk for tissue injury.

The evolutionary persistence of fever indicates that this strategy is usually effective. However, until twentieth-century man, increases in body temperature occurred almost exclusively during active infections or in situations associated with enhanced risk of infection, including traumatic injury, exertion, or flight or fight responses. In modern man, the acute phase response comprising fever and inflammation, is often activated by stimuli other than replicating pathogens, including antibiotic-treated infections and noninfectious causes.[8] We hypothesized that in such situations, the increased rate of host tissue injury stimulated by fever would not be counterbalanced by accelerated pathogen clearance, thereby causing harm rather than conferring protection.

We chose pulmonary oxygen toxicity, a highly clinically relevant, noninfectious form of injury that is unique to modern medicine to test this hypothesis. Exposure to artificially high concentrations of oxygen is often essential for patient survival during severe illnesses.[9] Unfortunately, such exposure is associated with severe, often lethal lung injury in man,[10] other primates,[11] and rodents.[12–22] The mechanisms of acute hyperoxic lung injury have not been definitively elucidated, but it is widely believed that generation of reactive oxygen species (ROS) plays a key role.[23] The contribution of neutrophils (PMN) to pulmonary oxygen toxicity is less clear. PMN recruitment to the hyperoxic lung occurs late in the course of lung injury.[24,25] Furthermore, PMN depletion produces inconsistent effects on hyperoxic lung injury with as many studies showing protection[19,26,27] as showing no effect[24,28–30] or even worsening of pulmonary oxygen toxicity.[24] The cytokines, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-3, and IL-6, as well as the chemokines monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, MIP-2, and γ-interferon-inducible protein (IP)-10[18,20,31] each accumulate, and expression of both adhesion molecules, intercellular adhesion molecule (ICAM)-1 and E-selectin increase[32] in the hyperoxic lung. Collectively, these studies suggest that despite its non-biological nature, hyperoxia appears to cause lung injury in part by activating the innate immune response.

We used a conscious, temperature-controlled mouse model to determine how co-exposure to FRH modifies pulmonary oxygen toxicity. We showed that maintaining core temperature between 39°C and 40°C (by increasing ambient temperature from 24°C to 34°C) rather than at normal basal levels (36.5°C to 37°C) in mice exposed to hyperoxia accelerates lethal pulmonary vascular endothelial injury, reduces the threshold of inspired oxygen level for lethality, induces expression of the PMN growth factor granulocyte-colony stimulating factor (G-CSF), and expands the circulating PMN pool. Furthermore, FRH was shown to augment pulmonary expression of the glutamic acid-leucine-arginine motif-positive (ELR^+) CXC chemokine genes KC and LPS-induced CXC chemokine (LIX), enhance CXC receptor-2 (CXCR2)-dependent recruitment of PMN, and change the histological pattern of lung injury to a neutrophilic interstitial pneumonitis.

Materials and Methods

Animal Exposure and Temperature Monitoring

Eight- to ten-week-old male outbred CD-1 mice, weighing 25 to 30 g were purchased from Harlan-Sprague Co. (Indianapolis, IN), housed in the Baltimore Veterans Administration Medical Center animal facility under the supervision of a full-time veterinarian, and used within 4 weeks of delivery. Mice were adapted to standard plastic cages for at least 4 days before study. To avoid the influence of diurnal cycling, all experiments were started at approximately the same time each day (between 8:00 a.m. and 10:00 a.m.). One week before each experiment, sentinel mice were implanted with telemetric intraperitoneal temperature sensors (Mini Mitter 1.05g *in vivo* temperature sensor model 100-0035; Bend, OR).

Experimental mice were housed in modified plastic cages to allow continuous inflow of air/oxygen mixtures and outflow through a 1 cm^2 outflow port. The oxygen level in each cage was measured twice daily using an oxygen analyzer (Vascular Technology; Chelmsford, MA). Groups of three to four mice, including one thermister-implanted sentinel mouse per group, were placed in each cage. The cages were placed in modified infant isolettes with temperature set to 24°C to maintain euthermia or 34°C to 34.5°C to maintain FRH and core temperatures of the sentinel mice were continuously monitored using the Mini Mitter Automated Data Acquisition System. Except for the ambient temperature, handling of the euthermic and hyperthermic mice was identical. Exposures to hyperthermia and hyperoxia were initiated simultaneously. All procedures were approved by the University of Maryland Baltimore Animal Care and Use Committee.

Lung Lavage and Blood Processing

At selected times, groups of mice were anesthetized by 10 to 30 second exposure to isoflurane, euthanized by cervical dislocation, and lung lavage was performed *in situ* through an 18g blunt-end needle secured in the trachea, using 1 ml phosphate-buffered saline (PBS) instilled and withdrawn twice, followed by instillation and recovery of a second 1 ml of PBS. The two aliquots of lung lavage were pooled, cells were collected by centrifugation at 1000 $\times$ *g* for 3 minutes, and cell-free supernatants were stored at -80°C for analysis of total protein and cytokine concentrations. Total cell counts were performed manually using a hemacytometer and differential cell counts of Diff-Quick-stained cytopreparations were performed by two blinded observers (PR and RMV) using morphological criteria. Heparinized blood was collected via cardiac puncture. Total blood PMN count was determined by manually counting total leukocytes after lysis of