ENDOTOXIN EXTENDS SURVIVAL OF ADULT MICE IN HYPEROXIA

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ABSTRACT. Research on endotoxin protection from oxygen toxicity is presently limited to the rat model since only rats have been protected by endotoxin. This study reports that endotoxin also extends survival of adult male mice in hyperoxia (> 99% oxygen at 1 ATA). Initially, 4-month-old male mice were treated with Boivin-extracted E. coli endotoxin and placed in hyperoxia. Zymosan-primed mice receiving 2 or 10 μg endotoxin, and unprimed mice receiving 10-40 μg endotoxin, showed moderate protection against hyperoxia; 11/15 Boivin-treated mice survived 120 hours exposure to hyperoxia with time-of-death in hyperoxia = 126.7 ± 4.4 hours (mean ± SEM, n=15). This contrasts with untreated male mice; 0/4 survived 120 hours exposure to hyperoxia with mean survival = 103.5 ± 3.5 hours. Mice receiving 20 or 60 μg Westphal-extracted endotoxin were not protected nor were older female mice receiving 20 μg Boivin-extracted endotoxin. This study suggests that age, sex, the extraction method used to obtain endotoxin, and possibly the time of year when endotoxin is administered, are important variables in allowing endotoxin to extend survival of mice in hyperoxia.

INTRODUCTION. In 1978 Frank, Yam and Roberts (1) first reported that endotoxin protects rats from oxygen toxicity. Since then, efforts to use endotoxin to protect mice against hyperoxic lung injury have failed (2-4). This lack of response in mice has led to the consensus that endotoxin protection against hyperoxia is species-specific (5, 6).

Recently, Hazinski et al. (7) reported that endotoxin prevents hyperoxic lung injury in lambs. This observation, and the fact that enhancement of the immune response with adjuvants is often required before many of the effects of endotoxin on mice are observed (8, 9) led us to reinvestigate endotoxin protection against hyperoxia in mice. This study reports that endotoxin extends survival of mice in hyperoxia but protection appears to be influenced by the age and sex of the animal, the season, and the type of endotoxin preparation used.

MATERIALS and METHODS. Endotoxin (Boivin-extracted Lipopolysaccharide B E. coli 055:B5, LD_{50}=13.31 mg/kg, and Westphal-extracted Lipopolysaccharide W E. coli 055:B5, LD_{50}=19.4 mg/kg) was obtained from Difco Laboratories (Detroit, Michigan). Zymosan A (# Z-4250) was purchased from Sigma Chemical Co. (St. Louis, MO.). Both endotoxin and zymosan were dissolved in sterile 0.9% sodium chloride and vortexed for 10 minutes prior to injection (0.5 ml injection volume) with endotoxin being administered immediately before oxygen exposure.

Mice were obtained from Charles River (CF-1 strain, Kingston, Stone Ridge, N.Y.) and maintained on standard laboratory chow and water ad libitum both before and during experiments. All mice were acclimatized to the laboratory for at least 15 days prior to use to insure full responsiveness to endotoxin (10).

Oxygen exposure: The exposure chamber (20"x 30"x7") was made of clear plexiglass and housed up to 18 mice/experiment in separate compartments. During hyperoxia mice were allowed food and water ad libitum and were supported by a raised, wire-mesh platform above wood shavings and soda lime, which lined the floor to absorb urine and CO_2. Oxygen concentration was maintained at > 99% (MiniOx I oxygen analyzer, Catalyst Research Corp., Owings Mills, MD.) and CO_2 levels < 0.2% (Beckman Medical Gas Analyzer LB-2). Dead mice were removed from the chamber through a 1.4" hole in the top which was sealed, during the experiment, by the water bottle. This procedure allowed removal of dead mice with only a momentary fall in chamber PO_2 (< 10% change overall).

Study #1 (designed to determine if enhancement of the immune response with zymosan is required to obtain endotoxin protection of mice from oxygen toxicity) used 4-month-old adult male mice (32-43 grams) and was conducted from Nov. 28 to Dec. 10, 1988. For priming (8), mice received 3 daily injections of zymosan (1 mg/0.5 ml via tail vein) and endotoxin was injected 48 hours later. Mice were allowed to remain in hyperoxia until death to determine survival time.

Study #2 (designed to determine whether the method used to extract endotoxin influences its ability to extend survival of mice in hyperoxia) employed a new batch of male mice and was conducted on Jan. 5-10, March 24-29, and March 31 to April 5, 1989. The mice used in Jan. (2 months old) weighed 28-38 grams; the mice used in March (4½ months old) weighed 35-48 grams.

Study #3 (designed to determine if the sex of the animal influences protection, and whether zymosan-priming would restore the ability of endotoxin to protect female mice from hyperoxia) used female retired breeders (8-9 months old, weighing 28-41 grams) and was conducted on April 14-19 and May 4-9, 1989. Mice were primed with

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zymosan according to the method of Carswell et al. (9); a single injection of zymosan (2 mg/0.5 ml, iv) preceeded the administration of endotoxin by 15 days. In this Study, and in Study #2, exposure to hyperoxia was discontinued at 120 hours and the number of surviving mice in each group noted.

Statistics: The significance ($p < 0.05$) of differences in time-of-death in hyperoxia between endotoxin-treated and No Treatment mice was evaluated by analysis of variance (ANOVA, Table I). The life table method (11) was used to test the significance of differences in 120-hour survival for mice receiving Boivin- or Westphal-extracted endotoxin (Table II).

Table I. Hours of Survival in Hyperoxia for 4-Month-Old Zymosan-Primed and Unprimed Male Mice Receiving Boivin-Extracted Endotoxin or No Treatment

A. Treatment	None	Zymosan-primed + endotoxin		
		2 μg	10 μg	20 μg
Survival (hr)	96	<24	106	<24
	100	119	116	<24
	106	127	122	<24
		127	122	82
		132	152	106
B. Treatment	None	Unprimed + endotoxin		
		10 μg	20 μg	40 μg
Survival (hr)	112	88	145	124
		137	155	129
Treatment	None	Primed and unprimed + endotoxin		
Group survival (hr)	103.5 ± 3.5 (4)	126.7 ± 4.4[a] (15)		

[a] Differences in survival (mean ± SE) between the group of zymosan-primed and unprimed mice receiving endotoxin at a protective dose and dying from oxygen toxicity, and the group receiving no treatment (none) are significant at $P < 0.50$ by ANOVA. Twenty micrograms of endotoxin appeared to be toxic to primed mice, so this group was excluded. Numbers in parentheses, mice.

RESULTS. Study #1: 15 zymosan-primed male mice were treated with Boivin-extracted endotoxin (2-20 μg) and placed in hyperoxia along with 3 untreated mice. Table I-A shows that 3/4 (75%) of the zymosan-primed mice receiving 2 μg endotoxin and dying from oxygen toxicity, and 60% of zymosan-primed mice given 10 μg endotoxin, survived exposure to hyperoxia for 120 hours while a dose of 20 μg endotoxin was toxic (3 of 5 zymosan-primed mice receiving this dose died from complications unrelated to oxygen toxicity within the first day). 8/9 (89%) of the primed mice receiving 2 or 10 μg endotoxin and dying from oxygen toxicity outlived the No Treatment group of mice and none of the untreated mice survived 120 hours in hyperoxia.

In the next experiment 6 unprimed mice (from the same batch of mice used earlier) were given Boivin-extracted endotoxin (10-40 μg) and placed in hyperoxia along with 1 untreated mouse (Table I-B). 5/6 (83%) of these mice survived 120 hours exposure to hyperoxia while the single untreated mouse in this group died at 112 hours exposure. Survival in hyperoxia for all 4 untreated mice averaged 103.5 ± 3.5 hours (mean ± SEM) while primed mice (receiving 2-10 μg endotoxin and dying from oxygen toxicity) and unprimed mice receiving 10-40 μg endotoxin survived hyperoxia for 126.7 ± 4.4 hours (n=15). The difference in time-of-death in hyperoxia between untreated mice and mice receiving Boivin endotoxin is significant at $p < 0.05$ by ANOVA. Zymosan-priming did not affect survival in hyperoxia; zymosan-primed mice (dying from oxygen toxicity and not endotoxemia) lived 124.8 ± 4.2 hours in hyperoxia (n=9) while unprimed mice survived 129.7 ± 9.5 hours (n=6). However, it is interesting to note that zymosan-priming altered the sensitivity of mice to endotoxin as observed by others (12); 20 μg endotoxin was toxic to primed mice but provided the best protection against hyperoxia in unprimed mice.

Mice, dying from hyperoxia, exhibited typical symptoms of oxygen toxicity; they developed severe respiratory distress shortly before death with intratracheal edema and liver-like lungs on post-mortem examination. Mice, dying from endotoxemia and not hyperoxia (i.e., those dying within 24 hours following injection with endotoxin), occasionally bled from the ears or nostrils and exhibited bright red lungs that were otherwise normal in appearance.

Study #2: Since optimal protection seemed

Table II. Hours of Survival in Hyperoxia in Unprimed Male Mice Receiving Boivin- or Westphal-Extracted Endotoxin

Treatment	None	Type of endotoxin		
		Boivin	Westphal	
		(20 μg)	(20 μg)	(60 μg)
Survival (hr)				
2-month-old mice	93, 119	106, 111, >120 (2)	94, 102, 107,115, >120 (1)	101, 110, 112, 115, >120 (1)
		Boivin		
		(20 μg)		
4½-month-old mice	96, 112, 118, >120 (1)		104, 107, 111, 116, >120 (6 mice)	

to occur when 20-40 μg Boivin endotoxin was given to unprimed mice (Table I-B) Study #2 was conducted using similar dosages. 14 male mice (2 months of age) were treated with Boivin- or Westphal-extracted endotoxin and placed in the chamber, along with 2 untreated mice. Table II shows that 2/4 (50%) of 2-month-old mice receiving Boivin endotoxin survived 120 hours exposure to hyperoxia while only 2/10 (20%) of the mice treated with Westphal endotoxin survived the exposure period. 2½ months later 10 more mice from the same group (now 4½ months old) were treated with 20 μg Boivin endotoxin and exposed to hyperoxia, along with 4 No Treatment mice. 4½-month-old mice (60% survival) are as well protected against hyperoxia by Boivin endotoxin as 2-month-old mice. Although mice receiving Boivin endotoxin tended to exhibit increased 120-hour survival in hyperoxia compared to mice receiving Westphal endotoxin (57% vs 20% survival, respectively) the difference between groups is not significant ($p > 0.05$). Further studies, using larger groups of mice, are required to resolve this point. Finally, 1/6 (16.7%) of No Treatment mice in this Study survived exposure to hyperoxia for 120 hours with 2 other mice surviving for 118 and 119 hours. When compared to the survival of No Treatment mice in Study #1 (Table I) it appears that survival of mice in hyperoxia may be influenced by season.

Study #3: 10 retired female breeders were treated with 20 μg Boivin endotoxin and placed in hyperoxia along with 3 No Treatment mice. Three weeks later a second group of 8 zymosan-primed female mice were treated with Boivin-extracted endotoxin and placed in hyperoxia along with 3 No Treatment mice. The collective data from these two experiments (Table III) shows that endotoxin did not increase survival of these older female mice in hyperoxia.

DISCUSSION. Study #1 provides the best evidence that endotoxin extends survival of adult male mice in hyperoxia. 11/15 (73%) of mice receiving a protective (and not toxic) dose of endotoxin survived exposure to hyperoxia for 120 hours. By comparison, none of the untreated mice in this group survived the same time period (Table I). Survival times in hyperoxia for mice in this study were; Endotoxin Treatment = 126.7 ± 4.4 hours (mean ± SEM) and No Treatment = 103.5 ± 3.5 hours ($p < 0.05$ by ANOVA).

Table III. Hours of Survival in Hyperoxia in Unprimed or Zymosan-Primed Female Mice Treated with Boivin-Extracted Endotoxin

Treatment	None	Unprimed + endotoxin (20 μg)		
Survival (hr)	95 96 116	86, 88, 92, 95, 100, 111, 112, 114, 119, >120 (1)		
		Zymosan-primed + endotoxin		
		0.2 μg	2 μg	10 μg
	105 105 115	100 105 114	89 105 105	88 105

Study #2 found that the extraction procedure used to isolate endotoxin appears to alter endotoxin's ability to extend survival in hyperoxic mice; 8/14 (57%) of mice receiving Boivin-extracted endotoxin survived 120 hours exposure to hyperoxia while only 2/10 (20%) of mice receiving Westphal-extracted endotoxin survived the same exposure period (Table II). Though preliminary, this observation suggests that those parts of the endotoxin molecule retained by Boivin-extraction but lost during Westphal-extraction (i.e., peripheral proteins and other acid soluble molecules) may participate in extending the survival of mice in hyperoxia. Extensive work by others has established structure/function correlations for the endotoxin molecule and associated components in numerous models (13, 14). The hyperoxic mouse model (though not fully defined in this report) may provide yet another tool for study in this interesting, and potentially important, area of research.

The ability of endotoxin to extend survival of rats in hyperoxia has been extensively studied (1-4) and appears to be more impressive than the effect on mice observed in this study. However, even in rats endotoxin protection against oxygen toxicity is not complete; Thet et al. (15) comment that the lungs of endotoxin protected rats at 60 hours exposure are similar in appearance to the lungs of unprotected rats at 40 hours exposure. It is likely that the model of endotoxin protection against hyperoxia is more complicated in the mouse than in the rat. Behling (16) reports that the timing of endotoxin injection is critical in protecting mice against irradiation. Mice, injected at 2 or 9 days before irradiation exhibit maximal protection; injections at other times provide little or no protection. Further work should lead to improved methods of protecting mice against hyperoxia with endotoxin.

In summary, Boivin-extracted endotoxin (in doses of 2-10 μg) in zymosan-primed mice or 10-40 μg (in unprimed mice) extends survival in hyperoxia. Although zymosan-priming rendered mice more susceptable to the toxic effects of endotoxin, it did not appear to enhance survival. Using results of survival to 120 hours, Westphal-extracted endotoxin may not be as effective in prolonging survival as Boivin-extracted endotoxin. These results were obtained in adult male mice. However, in older female mice no survival advantage using Boivin-extracted endotoxin was observed.

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