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Enhancement of the Lethal Effects of Endotoxins by Interferon Inducers

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Prior treatment of mice with poly I:C or NDV enhanced the lethal effect of *E. coli* or *B. dermatitidis* endotoxins.

It appears that interferon possesses a wider and more complex spectrum of activity than is now understood. For example, mice pretreated with interferon inducers exhibit a prolonged survival time after challenge with *Plasmodium berghei* (2, 5). Since phagocytosis may be involved in the resistance of mice to *P. berghei* (W. Cantrell and E. E. Elko, Fed. Proc. p. 139, 1964) and to endotoxins (4), it was deemed pertinent to study mouse endotoxemia after treatment with interferon inducers.

Newcastle disease virus (NDV) strain B1 was prepared as described previously (1). Synthetic double-stranded ribonucleic acid (RNA), polyinosinic-polycytidylic acid (poly I:C; Microbiological Associates, Bethesda, Md.), was diluted immediately before use in phosphate-buffered saline at *p*H 7.4. Swiss-Webster mice weighing 20 to 25 g were used through the study. Yeast cells of *Blastomyces dermatitidis* (Tonar isolate, obtained from E. S. McDonough, Marquette University, Milwaukee, Wis.) were grown in a peptone glucose broth (3), harvested by centrifugation, washed in saline, and killed with acetone. After the acetone was removed by centrifugation and evaporation under vacuum, the cells were washed in saline and broken in a French press and the sediment was recovered after centrifugation. The sediment was washed and suspended in merthiolated saline, 1:10,000 (v/v), and a dry weight determination was made. *Escherichia coli* lipopolysaccharide W (026:B6; Difco) was diluted to the appropriate concentration in physiological saline. *Mycobacterium smegmatis* (ATCC 14468), the adjuvant used with the *B. dermatitidis* endotoxin, was grown in Proskauer Beck broth (Difco), washed, resuspended in merthiolated saline, and sonically treated briefly, and a dry weight determination was made (2 mg/0.1 ml).

In the first series of experiments, 6×10^7 plaque-forming units in 0.4 ml were injected intravenously (IV) into two groups of animals. At 6 hr later, the first group was injected intraperitoneally (IP) with 1 ml of *B. dermatitidis* endotoxin immediately followed by 0.1 ml of adjuvant (IP), and the second group was injected IV with 0.1 ml of *E. coli* lipopolysaccharide. Untreated mice received similar injections of these endotoxins. Control groups included: (i) mice injected with NDV; (ii) NDV, merthiolated saline, and adjuvant; and (iii) NDV and 0.85% saline. Deaths were recorded daily for 3 days (Table 1). It is evident that the lethal effects of both endotoxins in mice are enhanced by prior injections of NDV. Deaths did not occur in the control groups.

An attempt was made to confirm the enhanced lethality of the endotoxins by using a chemical inducer, poly I:C, rather than a viral inducer of interferon. In the second series of experiments, 70 μg of poly I:C in 0.4 ml was injected IV into two groups of mice (Table 2). As in the previous series, these animals and two additional groups of untreated mice was injected with *B. dermatitidis* or *E. coli* endotoxins. The results show an enhanced lethality of the endotoxins attributable to prior injections of poly I:C. No toxic effect was observed in an additional group of mice treated only with 70 μg of poly I:C.

The lethal and interferon-inducing effects of bacterial endotoxins have been reported, and these two phenomena may be related (6, 8). Both endotoxin and poly I:C stimulate the release of interferon (7), and, after treatment of mice with lead acetate, there is an increase in the quantity of interferon released after the injection of endotoxin or poly I:C (W. R. Stinebring and M. Absher, *personal communication*). Therefore, the increase in endotoxin lethal effect after poly I:C treatment can be interpreted as additive. The implication is strong, however, that the enhanced lethality of endotoxins is mediated by interferon since NDV, which induces the syn-

TABLE 1. *Effect of NDV on susceptibility of mice*

Endotoxin	Dose of endotoxin	NDV-treated mice			Untreated mice		
		Cumulative no. of deaths/total at			Cumulative no. of deaths/total at		
		1 day	2 days	3 days	1 day	2 days	3 days
Blastomyces dermatitidis	83 mg	8/8			5/8	8/8	
	60 mg	9/10	10/10		2/10	6/10	6/10
	50 mg	10/12	10/12	10/12	2/12	2/12	2/12
	30 mg	4/10	8/10	8/10	0/10	1/10	1/10
Escherichia coli	300 μg	8/8			1/8	2/8	2/8
	150 μg	5/8	7/8	7/8	0/8	0/8	0/8
	50 μg	3/9	3/9	3/9	1/9	1/9	1/9

TABLE 2. *Effect of poly I:C on susceptibility of mice*

Endotoxin	Dose of endotoxin	Poly I:C-treated mice[a]			Untreated mice		
		Cumulative no. of deaths/total at			Cumulative no. of deaths/total at		
		1 day	2 days	3 days	1 day	2 days	3 days
Blastomyces dermatitidis	50 mg	6/10	7/10	7/10	1/10	1/10	1/10
	25 mg	1/10	1/10	1/10	0/10	1/10	1/10
Escherichia coli	250 μg	10/10			5/10	6/10	6/10
	150 μg	10/10			1/10	2/10	2/10
	100 μg	9/10	9/10	9/10	1/10	1/10	1/10

[a] Treatment was 70 μg of poly I:C in 0.4 ml IP.

thesis of interferon, also increases the lethal effect of endotoxin in mice. Studies are underway to support this hypothesis.

Since it is well known that interferon inducers protect against viral and parasitic infections in animals, we consider it significant that enhancement of lethality was observed after injection of endotoxins. The latter biological effect suggests that the use of these inducers as therapeutic agents may enhance the symptoms of infectious diseases in which endotoxin contributes to pathogenesis.

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LITERATURE CITED

1. Huang, K., and F. B. Gordon. 1968. Production of interferon in mice: effect of altered gaseous environments. Appl. Microbiol. 16:1551–1556.
2. Jahiel, R. I., J. Vilček, R. Nussenzweig, and J. Vanderberg. 1968. Interferon inducers protect mice against *Plasmodium berghei* malaria. Science 161:802–804.
3. Landay, M. E., E. P. Lowe, J. Mitten, and F. X. Smith. 1968. Disseminated blastomycosis in hamsters after intramuscular, subcutaneous and intraperitoneal injection. J. Intern. Soc. Human Animal Mycol. 6:318–323.
4. Nowotny, A. 1969. Molecular aspects of endotoxic reactions. Bacteriol. Rev. 33:72–98.
5. Schultz, W. W., K. Huang, and F. B. Gordon. 1968. Role of interferon in experimental mouse malaria. Nature (London) 220:709–710.
6. Suter, E. 1964. Hyperreactivity to endotoxin in infection, p. 435–447 *In* M. Landy and W. Broun (ed.), Bacterial endotoxins. Rutgers University Press, New Brunswick, N.J.
7. Youngner, J. S., and J. V. Hallum. 1968. Interferon production in mice by double-stranded synthetic polynucleotides: induction or release? Virology 35:177–179.
8. Youngner, J. S., and W. R. Stinebring. 1965. Interferon appearance stimulated by endotoxin, bacteria, or viruses in mice pretreated with *Escherichia coli* endotoxin or infected with *Mycobacterium tuberculosis*. Nature (London) 208:456–458.