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Studies on Nonspecific Acquired Resistance to Viral Toxicity in Mice.* (23303)

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Results obtained in several laboratories have indicated that prior injection of viable or inactivated influenza virus protects mice to challenge with toxic quantities of virus administered by the same route. The rapidity with which resistance develops, and its production by serologically heterologous virus, preclude the possibility that specific immunity based upon antibody production is involved in such protection. Such protection has been produced in mice both by intracerebral(1,2) and intravenous(3,4) routes and resembles viral interference. On the other hand, susceptibility of experimental animals to bacterial infection or endotoxin has been modified by a number of nonspecific physiological factors(5-8). For example prior injection of bacterial endotoxin protected mice against subsequent bacterial infection or challenge with endotoxin, and was associated with an elevation of the properdin level of the serum (6-7). The present investigation was undertaken to determine what relation, if any, these phenomena might have to one another.

Methods. The PR8 and NWS (neurotropic) strains of influenza A virus, the Lee strain of influenza B virus, and the RO strain of Newcastle disease virus (NDV) were cultivated in the allantoic sac of embryonated eggs. Virus was sedimented in a Spinco model L ultracentrifuge at 78,000 x g, resuspended in 1/10 the original volume of tryptose broth, and stored at –70°C. Technics for demonstrating the toxic effects of intracerebrally injected influenza viruses(9,10) and NDV(11,12) have been described. Protection tests in mice were performed similar to that described by Wagner(1). Weanling Swiss albino mice weighing 9-10 g each were used for intracerebral inoculation. Prechallenge injections were 0.01 ml, while challenge injections made into the opposite hemisphere were 0.03 ml. All intravenous injections were 0.5 ml into the tail vein of mice weighing 18-20 g each. All mice were observed daily for 5 days and necropsied when the experiment was terminated. *Escherichia coli* endotoxin was prepared by the trichloroacetic acid method(13). The LD_{50} of these preparations was approximately 0.18 mg when injected intravenously into 20 g mice.

Results. Table I summarizes the results of 3 experiments showing that prior intracerebral injection of a number of unrelated materials exerted a modifying effect on the neurotoxic effect of influenza A virus. Groups of 20 or 40 mice each were injected intracerebrally with the indicated amounts of ribonucleic acid (RNA), desoxyribonucleic acid (DNA), purified type III pneumococcus polysaccharide, *E. coli* endotoxin, undiluted horse serum, or with saline. RNA and DNA were adjusted to pH 6.8-7.0 with NaOH and were used, as was the pneumococcus polysaccharide, at the limit of solubility. Twenty-four hours later, half of the mice in each

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TABLE I. Nonspecific Resistance to Neurotoxicity of Influenza A Virus in Mouse Brain.

Material injected	ED_{50} of challenge virus: 2.7	5.4
	C/T	
−24 hr		
10 μg RNA	1/10	5/10
10 μg DNA	3/10	9/10
100 μg Type III pneumococcus polysaccharide*	1/ 9	5/ 9
Horse serum	6/10	8/10
Saline	15/22	20/22
−24 hr		
10.0 μg *E. coli* endotoxin	0/ 8	1/10
1.0 " "	2/10	1/10
.1 " "	0/10	3/10
.01 " "	0/10	9/10
Saline	11/12	12/13
−24 hr		
.1 μg *E. coli* endotoxin	2/19	15/20
Saline	18/19	20/20
−6 hr		
.1 μg *E. coli* endotoxin	3/19	8/20
Saline	17/20	16/16
+6 hr		
.1 μg *E. coli* endotoxin	19/19	18/18
Saline	18/18	18/19

C/T = No. of mice convulsing/total.
ED_{50} = 50% tonic convulsive dose.
−24 hr = 24 hr before virus.
+6 hr = 6 hr after virus.
* Kindly provided by Dr. Michael Heidelberger.

group were challenged intracerebrally with 2.7 ED_{50} (50% tonic convulsive doses) of influenza A virus and the remaining mice were injected with 5.4 ED_{50} of virus. Groups of mice were also treated with endotoxin at 24 and 6 hours before and 6 hours after challenge with lethal doses of influenza virus. Twice daily for 3 days following challenge, each mouse was suspended by its tail and twirled vigorously to induce convulsions. Each mouse that convulsed was killed, and the total number of mice convulsing was recorded, The results show that large doses of RNA, DNA, and pneumococcus polysaccharide afforded partial protection against 2.7 or more ED_{50} of influenza neurotoxin and that horse serum was without effect. *E. coli* endotoxin was most effective in protecting mice from the neurotoxicity of influenza virus. Indeed, as little as 0.01 μg of endotoxin protected mice against 2.7 ED_{50} of virus. Endotoxin was effective when given 6 or 24 hours before virus, but afforded no protection when given 6 hours after challenge.

It has been shown(12) that intracerebral injection of the RO strain of NDV in high dilution into mice causes paralysis and death in the absence of detectable viral synthesis. Prior intracerebral injection of influenza B virus or receptor destroying enzyme (RDE) protected mice against the toxic effect of highly diluted virus and delayed onset of symptoms following injection of concentrated virus. Heat-inactivated RDE delayed response to concentrated NDV, but had no effect on frequency or time to paralysis or death induced by high dilutions of NDV. Wagner (1) found that prior intracerebral injection of RDE or sublethal doses of influenza A virus or influenza B virus protected mice from intracerebral infection with the neurotropic NWS strain of influenza A virus. The effect of *E. coli* endotoxin on the response of mice to NDV and the NWS strain of influenza virus injected intracerebrally is shown in Table II. In 2 experiments, groups of 20 mice each were injected intracerebrally with appropriate quantities of endotoxin or with saline. Twenty-four hours later, half of the mice in each group were injected intracerebrally with 10x concentrated NDV (10^{+1} dilution) and the remainder were injected with NDV diluted 10^{-3}. Each mouse was examined daily, and the time of onset of the first unmistakable response (paralysis or prostration) or death was recorded. In a separate experiment, groups of 10 mice each were injected with dilutions of endotoxin or saline and each group was challenged 24 hours later with approximately 100 LD_{50} of NWS influenza virus. Mortality ratios were recorded and the average day of death was calculated for each group. The results show that prior injection of 0.001 μg of endotoxin more than doubled the latent period in mice challenged with concentrated NDV, but 100 μg of endotoxin had no effect on the response of mice to diluted NDV. Further, 10 μg of endotoxin failed to influence infection with NWS influenza virus.

Recently, Khoobyarian and Walker(3) showed that mice were protected from intra-

TABLE II. Effect of *E. coli* Endotoxin on NDV and Neurotropic Influenza (NWS) Virus in Mouse Brain.

1st inj.*	2nd inj.				
E. coli endotoxin inj. intracer. (μg)	RO-NDV 10^{+1} Exp. A	RO-NDV 10^{+1} Exp. B	RO-NDV 10^{-3} Exp. A	RO-NDV 10^{-3} Exp. B	NWS 10^{-2}
	ADR				
100.	3.9		5.3	5.2	
10.	3.9		5.9	5.9	6.9
1.	3.1		5.7	5.9	7.2
.1	3.4	3.5	6.3	6.4	7.1
.01	4.7	3.3	4.9	5.0	6.7
.001		3.0			
.0001		2.1			
Saline	1.5	1.4	6.4	6.0	6.5

* 10 mice/group.

ADR = Avg day of response. 10^{+1} = 10 × concentrated RO-NDV. NWS = NWS strain of influenza A virus.

venous toxicity of influenza virus by a prior intravenous injection of influenza A virus, influenza B virus, NDV, RDE, or heat-inactivated RDE. Prior injection of typhus rickettsiae, feline pneumonitis virus, or bacterial pyrogen in the form of typhoid vaccine were without effect. The data summarized in Table III show that a prior intravenous injection of *E. coli* endotoxin exerted a protective effect against the intravenous toxicity of influenza A virus. Groups of 20 or 40 mice each were injected intravenously with dilutions of influenza A virus, influenza B virus, NDV, *E. coli* endotoxin, or saline. Twenty-four hours later, groups of approximately 20 mice each were injected intravenously with 3 LD_{50} of influenza A virus or 1.4 LD_{50} of *E. coli* endotoxin. The mortality ratios of mice dying within 5 days without pulmonary consolidation were recorded for each group. It is clear that prior injection of endotoxin induced tolerance to challenge with lethal doses of influenza virus and endotoxin. However, prior injection of influenza A virus failed to protect mice against endotoxin. As anticipated(3,4), influenza A virus, influenza B virus, NDV, and RDE protected mice against the toxicity of influenza A virus.

TABLE III. Nonspecific Resistance to Viral Toxicity Produced in Mice by Intravenous Injection of *E. coli* Endotoxin.

1st inj.	2nd inj.	
	Influenza A virus	.25 mg endotoxin
	D/T	
Endotoxin, .1 mg	3/14	0/17
.08 "	4/16	
Saline	17/20	17/18
Influenza A virus: 10^{0}	2/19	17/17
10^{-1}	0/20	
10^{-2}	14/20	
Saline	18/20	11/20
Influenza B virus, 10^{0}	0/20	
Newcastle disease virus, 10^{0}	1/20	
Saline	37/37	

D/T = No. dead/total.

Extensive experiments, designed to demonstrate virus neutralizing factors in serum of mice that had received a protective dose of influenza A virus 24 hours previously, were unsuccessful. Further, prior intravenous injection of India ink and thorium dioxide failed to increase the resistance of mice to the toxic effects of influenza virus when barely tolerated amounts were administered 24 hours before challenge.

Discussion. The data presented confirm the fact that prior intravenous injection of sublethal quantities of bacterial endotoxin or influenza virus protected mice against subsequent challenge with the homologous agent. It is of particular interest that prior injection of endotoxin protected mice against subsequent inoculation of toxic quantities of influenza virus, while previous injection of influenza virus failed to protect mice against challenge with endotoxin administered by the same route. It would appear that the physiological mechanisms set into motion by intra-

venous injection of endotoxin were capable of protecting mice against challenge with both endotoxin and toxic quantities of influenza virus, while prior intravenous injection of influenza virus failed to elicit such a response. It should be emphasized that barely tolerated quantities of endotoxin were necessary to induce tolerance to either endotoxin or influenza virus. This fact may account for the recently reported(3) failure of typhoid vaccine to protect mice against the toxicity of influenza virus.

It is of interest that prior intracerebral injection of minute quantities of endotoxin (Table I), as well as sublethal quantities of virus(1,2), exerted a protective effect in mice challenged 24 hours later with toxic quantities of influenza virus by the same route, while endotoxin failed to protect mice against infection with the neurotropic NWS strain of influenza virus (Table II). Since prior intracerebral injection of non-neurotropic influenza virus has been shown to interfere with the neurotropic strain in mouse brain(1) and with the neuropathic effect of dilute RO-NDV(12) failure of endotoxin under similar conditions to induce resistance suggests that the mechanisms of viral interference and acquired resistance to viral toxicity are different. It is of interest in this connection to recall that Wagner(14) was unable to demonstrate an increase in the resistance of HeLa cells in tissue culture to the cytotoxic action of influenza virus after exposure of the cells to subtoxic quantities of the virus.

Summary. 1) Prior intravenous injection of *Escherichia coli* endotoxin or influenza virus protected mice against subsequent challenge with the homologous agent. Prior intravenous injection of endotoxin protected mice against subsequent inoculation of toxic quantities of influenza virus, while previous injection of influenza virus failed to protect mice against challenge with endotoxin administered by the same route. 2) Prior intracerebral injection of minute quantities of endotoxin, as well as sublethal quantities of influenza virus, exerted a protective effect in mice challenged 24 hours later with toxic quantities of influenza virus by the same route. Endotoxin failed to protect mice against infection with the neurotropic strain (NWS) of influenza virus or against the neuropathic effect of dilute Newcastle disease virus (RO strain). It is suggested that the mechanisms of viral interference and acquired resistance to viral toxicity are different.

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