MICROBIOLOGY

Patterns of Interferon Appearance in Mice injected with Bacteria or Bacterial Endotoxin

PREVIOUS work[1] has shown that the intravenous injection of large numbers of live *Brucella abortus* into chickens results in the appearance in the serum of a viral inhibitor with the properties of interferon. Inhibitor reached maximum levels about 12 h after inoculation. It was also reported that large numbers of Newcastle disease virus (NDV) particles produced maximum levels of interferon in chickens about 12 h after intravenous inoculation. Injection of large doses of other gram-positive and -negative bacteria or bacterial endotoxins failed to produce demonstrable inhibitors in the circulation of chickens at 6–8 h.

Since these results were reported, further work with chickens has shown that a barely detectable, but consistent, inhibitor activity appears in the serum at about 2 h after inoculation of large numbers of *Serratia marcescens* and *Salmonella typhimurium*, and after *Escherichia coli* endotoxin. In the case of *E. coli* endotoxin, it was necessary to inject birds intravenously with at least 40 mg in order to demonstrate inhibitor in serum at 2 h. Inhibitor activity, which usually was barely detectable at 2 h, had disappeared by 4–6 h after injection. The small and transient amounts in the serum made it too difficult to investigate the properties of the inhibitor produced at 2 h.

Table 1. VIRAL INHIBITOR IN PLASMA OF MICE AT DIFFERENT TIMES FOLLOWING INTRAVENOUS INJECTION OF VARIOUS ORGANISMS AND BACTERIAL LIPOPOLYSACCHARIDE

Material injected	Dose	Inhibitor titres* of plasma pools (hours post-injection)									
		0·5	1	1·5	2	3	4	6	12	14	16
Newcastle disease virus	2×10^7 plaque-forming units		<16		16		128	512	2,048	1,024	1,024
Brucella abortus	1×10^9 viable units	<16	<16		16			96	192	256	96
Escherichia coli 0111 : *B*4	250 μg	<16	128	256	768	256		128	24		
lipopolysaccharide†	25 μg				64			<16	<16		
Salmonella typhimurium	7×10^8 viable units	<16	64		384		192	16	<16		
Serratia marcescens	3×10^7 viable units	<16	128		256		32	16	<16		

* Reciprocal of the highest dilution of plasma giving 50 per cent inhibition of plaques of VSV in *L* cells.
† Nucleic acid not detected spectrophotometrically.

In extending these studies to other species, it was found that the intravenous injection of mice[2] with *B. abortus* or NDV resulted in significantly higher levels of circulating inhibitor than those found in chickens. This permitted us to examine the time pattern of appearance of viral inhibitor in mice as well as to compare the properties of the inhibitors produced in response to stimulation by different bacteria, NDV and endotoxin.

Swiss Webster female mice weighing about 25 g were injected in the tail vein with the different materials in a 0·1-ml. volume of saline diluent. Blood samples were obtained at different times from groups of about 10 mice by cardiac puncture with heparin-rinsed syringes. Pooled plasmas were assayed for viral inhibitor by the plaque-reduction method[3] using an interferon-sensitive line of *L* cells. Cultures of *L* cells in 60-mm Petri dishes were exposed for 20 h to 3 ml. of two-fold serial dilutions of plasma and then challenged with 40–60 plaque-forming units of vesicular stomatitis virus (VSV). The titres of viral inhibitors were calculated from the dilution of plasma which reduced the plaque count to 50 per cent of that of the controls. The dose of NDV was based on plaque-forming units; numbers of bacteria were given as viable units but it should be noted that approximately the same mass of bacteria, determined by optical density measurements, was employed in each case. Clumping or differences in size explain variations in viable counts.

Table 1 shows the inhibitor titres of mouse plasmas obtained at different times after the intravenous inoculation of several gram-negative bacterial species, *E. coli* 0111 : *B*4 endotoxin (Difco), and Newcastle disease virus. The results show that there were two distinct patterns of appearance of inhibitor in the circulation. The injection of NDV or *B. abortus* resulted in maximum inhibitor activity at 12–14 h. In contrast, the injection of *S. marcescens*, *S. typhimurium*, or endotoxin was followed by the early appearance of inhibitor in the plasma; maximum levels, far greater than any obtained in chickens, were observed at 2 h and these rapidly declined. Preliminary experiments have shown that direct exposure of *L* cells to 100 μg of endotoxin in serum-containing medium for 20 h did not reduce the subsequent plating efficiency of VSV, indicating that residual endotoxin in the plasma probably played no part in our experiments.

The characteristics of early-appearing inhibitor produced by endotoxin and the late-appearing inhibitors resulting from inoculation of *B. abortus* and Newcastle disease virus in mice were studied. The results showed that all three inhibitors prevented plaque formation by vesicular stomatitis virus only in mouse cells, not in chick embryo cells, and did not neutralize virus directly but acted on the host cells. In addition, all three inhibitors were stable to *p*H 3 treatment overnight but showed loss of activity when incubated with crystalline trypsin (50 μg/ml.) or when heated at 56° C for 1 h. These observations lead to the conclusion that the properties of the early-appearing inhibitor produced by endotoxin and the late-appearing inhibitors stimulated by *B. abortus* and NDV are similar to those of virus-induced mouse serum interferon as reported by Merigan[4].

The results with mice indicate that, as in chickens[1], *B. abortus* stimulated the appearance of interferon which reached peak titres in about 12 h. In mice, *S. typhimurium* *S. marcescens* and endotoxin produced inhibitor which appeared much earlier than inhibitor stimulated by *B. abortus* or NDV. This early-appearing inhibitor also resembled in properties and time of appearance the 'serum sparing substance' of Gledhill[5]. It is possible that the early-appearing interferon may be preformed and released from the cells with which the stimulating agent reacts, while the late-appearing interferon may represent inhibitor synthesized in response to *B. abortus* or NDV. Other explanations, including differences in the type of cell affected or differences in the rapidity of cell damage produced by the different agents, must be considered. However, it is known that all the agents used, including endotoxin[6,7], are rapidly sequestered in cells of the reticulo-endothelial system and it is possible that the inhibitors produced by the different agents are released in response to damage or stimulation of these cells.

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