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Interferon Induction and Resistance to Virus Infection in Mice Infected with *Brucella abortus*

A. BILLIAU,[1] E. SCHONNE, L. EYCKMANS, AND P. DE SOMER
Rega Institute for Medical Research, University of Leuven, 3000 Leuven, Belgium

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Induction of circulating interferon and protection against vaccinia virus infection in mice by injection of *Brucella abortus* were studied. It was demonstrated that morphologically intact brucellae (either live or killed by heat or exposure to NaOH) induce high and prolonged levels of circulating interferon in mice. In each instance, the inducing principle remained associated with the bacterial particle. Disruption of brucellae by mechanical means destroyed the interferon-inducing capacity. However, by alkalinization of the water extract of disrupted bacilli, an interferon inducer could be rescued. On intravenous injection, this inducer caused a typical endotoxin type of interferon response with a peak value at 2 hr. Mice pretreated with cycloheximide showed an enhanced interferon response to the brucella extract, but a reduced reaction to live brucellae. The significance of these data, in relation to the triggering of de novo interferon synthesis by brucella, is discussed. It was also observed that small doses of brucellae protected mice for at least 1 month against vaccinia virus infection. High doses of heat- or alkali-killed brucellae protected the animals for only a short time, and disrupted brucellae did not afford any protection. Thus, there was a good correlation between interferon-inducing capacity and short-term protective activity. Long-term protection, on the other hand, seemed to be related to multiplication of brucellae in the body.

Several bacteria have been reported to stimulate the appearance of circulating interferon after intravenous or intraperitoneal injection into animals (9). The failure of metabolic inhibitors to prevent this appearance of circulating interferon (5, 10, 13) has led to the concept that it is caused by a release of preformed interferon, not by de novo synthesis. In contrast, the interferon produced after stimulation with viruses, equally on the basis of studies with metabolic inhibition, is considered as synthesized de novo. In the present study the terms "inducer," "induction," and "induce" will be used to designate either of the two types of response. For most gram-negative organisms, the interferon-inducing properties are associated with the lipopolysaccharide component of the bacterial wall. Induction by intact bacteria and by extracted endotoxins follows the same pattern; i.e., circulating interferon attains a peak value at 2 hr postinjection and then abruptly decreases to zero again within a few hours. Intact brucellae, in contrast to enterobacteria, induce a more prolonged response and much higher interferon levels in the blood (8, 10, 12, 13; De Somer et al., Ann. N.Y. Acad. Sci., *in press*), and the induction is blocked by pretreatment of the test animals with cycloheximide (10, 13). The endotoxins from brucellae either are inactive (De Somer et al., *in press*) or induce a typical short-lived endotoxin-type response which is not blocked by cycloheximide (10, 13). Clearly, brucellae must contain a second inducer or there must be a physiological basis for the peculiar induction pattern of intact brucellae. In a previous study (De Somer et al., *in press*), we excluded the possibility that the active molecule is a nucleic acid. It was found that the inducing capacity of brucella is not separated by phenol-water extraction and is resistant to alkali. When entire brucellae were shaken in organic solvents, no lysis occurred, but the inducing capacity was strongly reduced. Recombination with the alcohol extract led to partial restoration of the original inducing potency. This would be consistent with the inducer's being a lipid-containing polymer.

In the present paper, we describe further experiments designed to clarify the mechanism whereby the intact brucella is a potent inducer and its

[1] "Bevoegderklvaard navorser" of the Belgian "N.F.W.O."

extracts retain only a limited or undetectable inducing capacity. Since it has been shown that *Brucella abortus* can protect animals against experimental viral infection (3), we also compared the ability of several brucella preparations to reduce lesion development in mice inoculated with vaccinia virus.

MATERIALS AND METHODS

The experimental animals were female NMRI mice weighing 20 to 25 g for experiments on interferon induction and 10 to 14 g for vaccinia tail lesion tests.

B. abortus (strain 19) cells were obtained as a lyophilized vaccine preparation from R.I.T., Genval, Belgium. The bacteria were disrupted in an ultrasonic vibrator (MSE, London, England) operating at 21 kc/sec or by shaking with glass beads (diameter, 0.10 to 0.11 mm) in a tissue homogenizer (Braun, Melsungen, West Germany). Disruption was monitored by microscopic examination. A phenol-extracted lipopolysaccharide from *B. abortus* (1) was kindly provided by F. M. Berger (Wallace Laboratories, Cranbury, N.J.). Cycloheximide was purchased from Sigma Chemical Co., St. Louis, Mo.

For induction of circulating interferon, groups of three mice were injected intravenously with bacterial or viral preparations. The animals were bled by puncture in the orbital sinus. Separate groups were used for bleeding at 2 and 6 hr after injection of the brucella preparations.

Resistance against viral infection was studied with the vaccinia tail lesion test (2). In practice, groups of 12 mice were injected intravenously with different brucella preparations. At times indicated in the text, vaccinia virus, obtained as a calf lymph suspension from the State Vaccine Office (Brussels, Belgium), was given intravenously in a dose calculated to cause the appearance of 20 pox lesions on the tail. The lesions were counted on day 7 after vaccinia virus administration.

Interferon titrations were done by use of the vesicular stomatitis virus (VSV) yield reduction assay. Specifically, 0.5 $\log_{10}$ dilutions of serum were put on duplicate mouse embryo fibroblast cultures and incubated for 24 hr at 37 C. The cultures were decanted and challenged with VSV at an input multiplicity of 10 plaque-forming units (PFU) per cell. The virus was left to adsorb for 1 hr, after which excess virus was removed by three washes. The cultures were refed and further incubated for 24 hr, at which time they were harvested. Newly produced VSV was quantitated by a plaque assay on L-929 cells. Virus yields (as $\log_{10}$ PFU/ml) were plotted against the $\log_{10}$ of the dilution of virus. A reduction of 0.5 $\log_{10}$ of virus yield was taken as the titration end point. In each titration, a reference mouse serum interferon was included. This permitted correction of individual values for variations of the sensitivity of the assay system and expression of values in terms of the internationally accepted NIH unit of mouse interferon (31st Symposium of the International Association of Microbiological Societies Permanent Section for Microbiological Standardization, London, 1969, *in press*).

RESULTS

Effect of heat treatment on interferon-inducing ability of B. abortus. When gram-negative organisms such as *Escherichia coli* or *Proteus rettgeri* are heated at 80 C, the endotoxin is released in the supernatant fluid (7). These endotoxins induce interferon when injected in vivo (4). Both the yields and time pattern are identical to those obtained with the entire live or heat-killed organisms. Thus, it seemed possible that the interferon-inducing principle would be released from *B. abortus* by a similar heat treatment. A brucella suspension (2×10^{10} live bacteria/ml) was heated at 80 C for 60 min. It was then centrifuged at 45,000 $\times$ *g* for 10 min. The clear supernatant was removed, and the pellet was suspended in the original volume of saline. Samples of the brucella suspension were also taken both before and after heating. Each fraction was tested for interferon induction in mice as described in Materials and Methods; the amount injected corresponded to 10^{10} live bacteria. The results (Table 1) showed that heating at 80 C, to a certain extent, reduced the interferon-inducing potency of the bacteria. However, the typical induction pattern of *Brucella* was preserved, i.e., high yields were obtained at

TABLE 1. *Induction of circulating interferon by heat- or alkali-killed brucellae*

Expt. no.	Injected prepn[a]	Circulating interferon (units/ml)	
		2 hr	6 hr
1	Intact brucellae	10,000	6,300
	Heated brucellae[b]	630	1,600
	Supernatant of heated brucellae[b]	80	32
	Pellet of heated brucellae[b]	2,200	6,300
2	Intact brucellae	2,500	10,000
	Heated brucellae[c]	12,500	2,000
	Heated[c] + NaOH-treated[d] brucellae	3,200	6,300
3	Intact brucellae	125,000	80,000
	NaOH-treated brucellae[d]		
	Total	63,000	5,000
	Pellet	6,300	8,000
	Supernatant	200	160

[a] Dose of injected material: equivalent of 10^{10} viable units of brucellae per mouse, intravenously.

[b] Heated at 80 C for 60 min. Supernatant separated from pellet by centrifugation at 45,000 $\times$ *g* for 10 min.

[c] Heated at 65 C for 30 min.

[d] NaOH (0.1 N), 37 C, 16 hr, followed by neutralization with HCl.

TABLE 2. *Effect of mechanical disruption on interferon-inducing potency of brucellae*

Expt. no.	Injected prepn	Circulating interferon (units/ml)	
		2 hr	6 hr
1[a]	Intact brucellae	50,000	16,000
	Disrupted brucellae[b]	≤500	500
	Supernatant of disrupted brucellae	<500	<500
	Pellet of disrupted brucellae	<500	<500
2[a]	Intact brucellae	2,000	80,000
	Disrupted brucellae[c]	16	16
3[d]	Intact brucellae	1,600	1,250
	Heat-killed brucellae[e]	2,000	2,000
	Heat-killed[e], disrupted[b] brucellae	40	<10

[a] Dose of injected material: equivalent of 10^{10} live brucellae per mouse, intravenously.

[b] Disruption by shaking with glass beads; pellet separated from supernatant by centrifugation at 45,000 × *g* for 20 min.

[c] Disruption by ultrasound.

[d] Dose of injected material: equivalent of $10^{9.7}$ live brucellae per mouse, intravenously.

[e] Heated at 80 C for 30 min.

both 2 and 6 hr after injection. Only a negligible fraction of the inducer was released into the supernatant medium.

Effect of mechanical disruption of B. abortus on its inducing potential. Disruption of bacteria was performed either by exposure to ultrasound or by shaking the bacteria with glass beads. Specifically, a suspension of live brucellae (2×10^{10} organisms/ml) was shaken for 3 min. The shaking flask was cooled by a stream of CO_2-snow. The cell debris could easily be decanted from the rapidly sedimenting glass beads. The beads were washed once, and the supernatant was combined with the first harvest of bacterial debris. Microscopically, no residual intact organisms were detectable. The cell walls were separated from the cytoplasm by centrifugation at 45,000 × *g* for 20 min. Samples of each fraction (intact brucellae, total disintegrated bacteria, cell walls, and cytoplasm) were tested for interferon-inducing capacity as indicated in Materials and Methods. Unexpectedly, it was found that mechanical disruption resulted in a nearly complete loss of interferon-inducing ability (Table 2). This result was confirmed by a second type of experiment in which the brucellae were disrupted by ultrasound at 0 C for 2 hr. Again, microscopic examination revealed no intact organisms in the suspension, and the equivalent of 2×10^{10} cells was injected in mice. Table 2 shows that, whereas the intact bacteria induced very high levels of interferon, the disrupted cells caused only a minute response.

Mechanical disruption may enhance autolysis of certain bacterial components. The inducer could thus be enzymatically degraded. The following experiments were designed to test this hypothesis. Brucellae were heated at 65 C, with the purpose of inactivating possible autolytic enzymes. Mechanical disruption of such heated cells likewise destroyed their inducing capacity (Table 2). Moreover, when intact brucellae were incubated for 2 hr at room temperature with a water extract of disrupted bacteria, no inhibition of the interferon-inducing ability was observed (Table 3). These negative results did not support the proposed hypothesis, but did not exclude it either, because autolytic activity may have escaped destruction in the first experiment and may not have been efficiently extracted in the second one.

TABLE 3. *Interferon induction by combined injection of intact and disrupted brucellae*

Expt. no.	Injected material	Circulating interferon (units/ml)	
		2 hr	6 hr
1	Intact brucellae[a]	80,000	32,000
	Disrupted brucellae[a]	30	<30
	Intact + disrupted brucellae[b]	3,200	4,000
2	Intact brucellae[c]	8,000	12,500
	Intact brucellae + supernatant of disrupted brucellae[d]	12,500	10,000
3	Intact brucellae[c]	—	10,000
	Disrupted brucellae[c]	—	<30
	Disrupted brucellae followed by intact brucellae[e]	—	3,200

[a] Equivalent of $10^{9.7}$ viable brucellae per mouse intravenously.

[b] Mixture of $10^{9.7}$ disrupted brucellae plus $10^{9.7}$ live brucellae per mouse intravenously. Mixture was incubated for 2 hr at room temperature before injection.

[c] Equivalent of 10^{10} live brucellae per mouse intravenously.

[d] Mixture of 10^{10} intact brucellae plus supernatant of 10^{10} disrupted bacteria per mouse intravenously. Mixture incubated for 2 hr at room temperature before injection.

[e] Disrupted brucellae (10^{10} per mouse) given intravenously 2 hr before 10^{10} live brucellae. Animals bled 6 hr after last injection.

Another explanation for the loss of activity in disrupted brucellae may be that a substance responsible for the induction of a hyporeactive state (10) is released and renders the mice refractory before the inducer can have its effect. To test this possibility, disrupted and entire brucellae were injected in the same animal, either simultaneously or with a time interval of 2 hr. The slight depression which was observed (Table 3) cannot be held responsible for the nearly complete inability of mice to respond to disrupted brucellae only. It may, for example, be explained by a competition of inactive cell wall fragments and active *Brucella* cells for the interferon-producing sites.

Influence of alkaline extraction on inducing potential of B. abortus. Exposure of brucellae to strong alkali does not destroy their interferon-inducing capacity (De Somer et al., *in press*). This is illustrated by the data of Table 1. A suspension of brucellae (10^{11} viable organisms/ml) was heated at 56 C for 30 min to kill the cells. Sodium hydroxide was then added to a final concentration of 0.1 N. After incubation at 37 C for 16 hr, the suspension was neutralized by the addition of HCl. When examined by phase microscopy, the bacterial cells could not be distinguished from viable organisms. Live cells, heat-killed cells, and heat-killed and NaOH-treated cells induced comparable amounts of interferon. Table 1 also shows that, after exposure to alkali, the inducer remained associated with the bacterial particles, and only a small fraction was released into the solution. Since this may have been due to the fact that brucellae possess a rather rigid and impermeable cell wall, a further attempt was made to obtain the inducer in solution by first disrupting the bacteria and then extracting them with alkali.

A suspension of brucellae (2×10^{10}) was homogenized by shaking with glass beads. A sample of the homogenate was exposed to NaOH (0.1 N final concentration) for 16 hr at 37 C, and then was neutralized. The rest of the homogenate was separated into membranes and supernatant by centrifugation at 3,500 $\times$ *g* for 30 min. The pellet was resuspended in the original volume. Both membranes and supernatant were then alkalinized for 16 hr at 37 C, neutralized, and again separated into a pellet and a supernatant. Each fraction thus obtained was tested for its interferon-inducing capacity in mice. The results, as summarized in Table 4, confirm that mechanical disruption results in an inactive suspension of cell debris. By exposing this suspension to alkali, an interferon-inducing material was obtained. Comparison of the activities of each fraction revealed that the masked inducer was present in the supernatant of the disintegrated bacteria and remained in solution after exposure to alkali. It can also be seen, however, that interferon induction by NaOH-extracted inducer followed the endotoxin pattern, i.e., a low-level response with a peak at 2 hr.

TABLE 4. *Extraction of an interferon inducer from disrupted brucellae by exposure to NaOH*

Injected prepn[a]	Circulating interferon (units/ml)	
	2 hr	6 hr
Intact brucellae	1,250	50,000
Disrupted brucellae	<40	<12
Disrupted brucellae treated with NaOH[b]	800	40
Pellet of disrupted brucellae treated with NaOH[b]		
Pellet	<12	<12
Supernatant	40	20
Supernatant of disrupted brucellae treated with NaOH[b]		
Pellet	12	<12
Supernatant	1,250	<12

[a] Dose: equivalent of 10^{10} viable units of brucellae per mouse intravenously.

[b] NaOH, 0.1 N, 37 C, 16 hr, followed by neutralization with HCl.

Effect of cycloheximide on interferon responses to B. abortus, to extracts prepared from B. abortus, and to other inducers. Induction of interferon by live brucellae was reported to be inhibited by cycloheximide, whereas endotoxins, either from enterobacteria or from brucellae, are potentiated by cycloheximide (10, 11, 13). Hence, it seemed logical to compare induction of interferon by brucellae and by the NaOH-extracted material in normal and cycloheximide-treated mice. Groups of three mice were given either saline or 5 mg of cycloheximide intraperitoneally 1 hr before induction with intravenous Newcastle disease virus (NDV), live brucellae, a phenol-extracted lipopolysaccharide from brucellae, or the NaOH-extracted interferon inducer from brucellae. The animals were bled 2 and 6 hr after injection of the inducers. The results (Table 5) clearly show that cycloheximide strongly reduced the interferon response to live NDV or brucellae but potentiated induction by the NaOH extract. The phenol-extracted lipopolysaccharide from brucellae was completely inactive in normal mice, as was found by R. H. Gustafsson and F. M. Berger (*personal communication*), but induced high interferon levels in cycloheximide-treated mice.

In vivo protection against vaccinia virus in mice injected with B. abortus. Infection of mice with *B.*

TABLE 5. *Influence of cycloheximide on the induction of circulating interferon by NDV, P. rettgeri endotoxin, B. abortus, and extracts from B. abortus*

Inducer	Dose per mouse (intravenously)	Cycloheximide (5 mg/mouse intraperitoneally)[a]	Circulating interferon (units/ml)	
			2 hr	6 hr
NDV	10^{10} EID_{50}	+	5	6,300
		−	25,000	125,000
B. abortus	10^{10} viable cells	+	<3	2,000
		−	5,000	3,200
Lipopolysaccharide from *B. abortus* (phenol-extracted)	200 μg	+	<20	200
		−	<20	<20
	100 μg	+	<20	2,500
		−	<20	<20
NaOH extract from *B. abortus*	Equivalent of 2×10^{10} cells	+	12	>80,000
		−	100	<20
	Equivalent of 10^{10} cells	+	400	4,000
		−	<20	<20
None	—	+	0	20

[a] Administered 1 hr before inducer.

abortus renders the animals resistant to infection with vaccinia virus (3). The following experiments were designed to investigate a possible correlation between protection against virus and induction of the interferon mechanism by different brucella preparations.

In a first experiment, we determined the dose and time relationships of protection afforded against vaccinia virus by live brucellae. Groups of 12 mice were injected intravenously with different doses of brucellae, and 1, 7, 14, and 29 days later the animals received vaccinia virus in a dose calculated to induce about 20 pox on the tail. The results (Fig. 1) show that the protective activity of brucella increased with time. Indeed, $10^{7.0}$ brucellae per mouse were necessary to cause a 50% reduction in pox lesion count when the vaccinia virus was given 1 day after the brucellae. The 50% protective dose decreased to $10^{6.5}$, $10^{4.0}$, and $10^{4.0}$ when the vaccinia virus was given 7, 14, and 29 days, respectively, after the brucellae. Similar experiments were carried out with brucellae killed by heat (65 C, 30 min), exposure to NaOH (0.1 N, 16 hr at 37 C), or mechanical disruption. From the results in Table 6, it appears that heated and alkali-treated brucellae partially retain their ability to protect against vaccinia virus given 1 day later. When the virus was given 7 days after the brucellae, the lesion scores were barely or not at all influenced by these types of brucella preparations. Disrupted brucellae did not protect the mice at all. Thus, short-term protection by killed brucellae correlates well with their ability to induce interferon. Indeed, heated or alkali-treated bacilli induced high levels of circulating interferon and also protected against pox development; disrupted brucellae, on the other hand, did not induce interferon and did not protect either. None of the preparations of dead brucellae could afford protection over a long period. This, together with our observation that protection afforded by small inocula of live brucellae increased with time, suggests that survival or even multiplication of brucellae in the organism is needed for the occurrence of long-

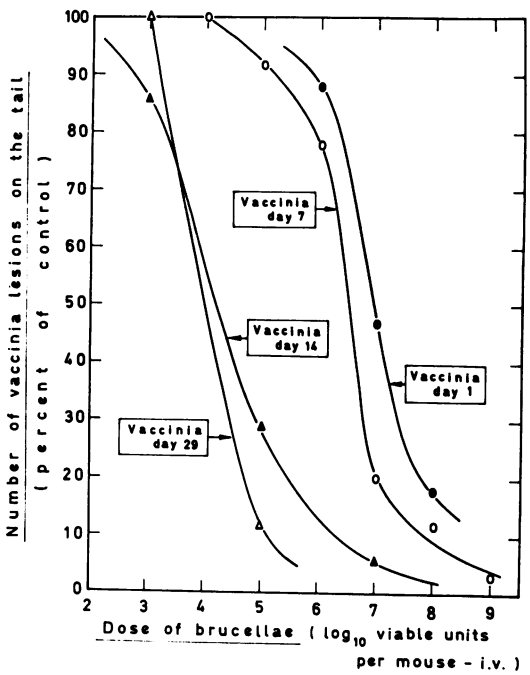

FIG. 1. *Inhibition of the development of vaccinia virus-induced pox lesions on the tail of mice by administration of B. abortus.*

TABLE 6. *Effect of different brucella preparations on the development of pox lesions on the tail of mice challenged with vaccinia virus intravenously*

Injected prepn	Dose (equivalent of viable brucellae per mouse)	Avg no. of pox lesions on the tail (% of control group)[a]	
		Vaccinia virus at day 1	Vaccinia virus at day 8
Heat-killed brucellae (65 C, 30 min)	10^9	35,15[b]	60
	10^7	65	—
NaOH-treated brucellae (0.1 N, 37 C, 16 hr)	10^9	22,50[b]	80
	10^7	43	—
Disrupted brucellae	10^9	71,86[b]	122
	10^7	82	

[a] Only reductions of $\geq 50\%$ in pox lesion count are statistically significant at the $P < 0.05$ level.

[b] Figures indicate values obtained in two separate experiments.

term protection. Control experiments, not reported in detail in the present paper, revealed that brucellae inoculated intravenously at a dose of 10^9 viable units per mouse survived in the spleen for more than 40 days. Seven days after infection with 10^4 viable units, multiplication of the bacteria in the spleen became evident.

DISCUSSION

After intravenous or intraperitoneal injection of brucellae in mice, high levels of circulating interferon can be detected (8, 10, 12, 13; De Somer et al., *in press*). Peak activity occurs after 6 to 12 hr and is maintained for several hours. Youngner, who reported on interferon induction by a lipopolysaccharide extracted from brucellae (10), did not specify which type of endotoxin was used, but he stated that the peak of circulating interferon occurred at 2 hr, compared with 6 to 12 hr for intact brucellae; moreover, induction was enhanced by pretreatment of the test animals with cycloheximide, whereas induction by intact brucellae was reduced by cycloheximide (10, 13). Thus, brucella endotoxin behaves as a regular endotoxin from enterobacteria. The question may then be raised whether the prolonged virus-type interferon response elicited by intact brucellae is caused by the endotoxin, or whether another inducer is present in the intact bacteria which is destroyed or neutralized during extraction procedures. Therefore, we attempted to extract *B. abortus* in different ways. Phenol extraction invariably resulted in a complete loss of activity, treatment of the bacteria with ethyl alcohol yielded an inactive extract and a poorly active precipitate, and trichloroacetic acid extraction again caused a loss of most of the activity (De Somer et al., *in press*).

Interferon-inducing lipopolysaccharides can be extracted from enterobacteria by heating at 80 C. When this kind of treatment was applied to *B. abortus*, the interferon-inducing ability was left intact (De Somer et al., *in press*). However, all of the activity remained associated with the bacilli and none was released into the medium. Exposure to 0.1 N NaOH also left the interferon-inducing ability of *B. abortus* intact; likewise, only a minute proportion of the activity was released into the supernatant fluid. Clearly, these treatments did not allow extraction of the inducing principle. It should be remarked that during exposure to heat or to alkali the bacterial cells were not lysed. It seemed indicated therefore to disrupt the cells by simple mechanical means before further extraction was attempted. Unexpectedly, it was found that mere disruption, by exposure to ultrasound or by shaking the bacteria with glass beads, resulted in a nearly complete loss of interferon-inducing capacity.

This may be due to destruction of the inducer by autolytic enzymes released during disruption of the brucellae, but no evidence for the existence of such a mechanism could be obtained. The failure of disrupted brucellae to induce high titers of interferon might also be due to the early development of hyporeactivity. Experiments in which mice were given combined injections of disrupted and live brucellae seemed to refute this hypothesis. Finally, the inability of disrupted brucellae to induce high and prolonged titers of circulating interferon might be explained on a physiological basis. It may be, for example, that several components and structures in *Brucella* have to be present in interferon-producing cells to elicit the typical long-term response. When disrupted bacteria are injected, the different components may be taken up by different cells.

From the water phase of homogenized brucellae, an interferon-inducing material could be extracted by exposure to NaOH. Circulating interferon obtained after injection of this inducer followed an endotoxin-type interferon pattern; low titers peaking at 2 hr were obtained. Moreover, pretreatment of the mice with cycloheximide enhanced the response to the NaOH-extracted material, whereas it inhibited induction by intact brucellae. Similarly, a phenol-extracted lipopolysaccharide from *B. abortus* induced measurable titers of interferon in cycloheximide-treated mice, but it seemed inactive in control animals. These experiments, which confirm earlier reports by Youngner and co-workers

(10, 13), can best be interpreted as indicating that *B. abortus* has the potential to elicit both the release of "preformed" interferon and de novo synthesis of interferon. Whatever the role of the brucella endotoxin in either mechanism may be, our results strongly suggest that a bacterial structure, sensitive to mechanical disruptive forces, must reach the relevant cells for de novo synthesis of interferon to be triggered. Whether this structure is only a macromolecule or a more complex morphological entity cannot at present be ascertained.

As described earlier, intravenously injected intact brucellae can protect mice against infection with vaccinia virus (3). The experiments reported here show that this protection lasts for at least 1 month. Heat- or alkali-killed bacteria protected mice for only a short period, and much higher doses were needed, suggesting that long-term protection depends on the survival or multiplication of *Brucella* in the host. This would also be consistent with the observation that the protective effect of a small dose of live brucellae was manifested only when vaccinia virus was given 2 or 4 weeks later.

The mechanism of resistance elicited by certain microorganisms against phylogenetically unrelated agents is a matter of conjecture (1, 3, 6). As far as viruses are concerned, the effect might be mediated by interferon. This would be consistent with the observation that a number of brucella preparations which induce interferon [e.g., live, alcohol-precipitated (De Somer et al., *in press*), heat-killed, or alkali-treated brucellae] did elicit at least short-term resistance against virus, whereas mechanically disrupted brucellae neither induced interferon nor protected the mice against the development of vaccinia lesions. Also, a phenol-extracted lipopolysaccharide (1) which induced interferon only in cycloheximide-treated mice did not protect the animals against challenge with Columbia SK virus (F. M. Berger and R. H. Gustafsson, *personal communication*).

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