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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: 2
2. GOVT ACCESSION NO.:
3. RECIPIENT'S CATALOG NUMBER:
4. TITLE (and Subtitle): Actions of Interferons on Macrophages
5. TYPE OF REPORT & PERIOD COVERED: Annual Report 6/1/84-5/31/85
6. PERFORMING ORG. REPORT NUMBER:
7. AUTHOR(s): Edward A. Havell, Ph.D.
8. CONTRACT OR GRANT NUMBER(s): N00014-83-C-0407
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Trudeau Institute, Inc., P.O. Box 59, Saranac Lake, NY 12983
10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS:
11. CONTROLLING OFFICE NAME AND ADDRESS: Jeannine A. Majde, Ph.d., Scientific Officer, Immunology Code 441, Cellular Biosystems Group, Department of the Navy, ONR Arlington, VA 22217
12. REPORT DATE: 6/1/85
13. NUMBER OF PAGES: 12
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office):
15. SECURITY CLASS. (of this report): Unclassified
15a. DECLASSIFICATION/DOWNGRADING SCHEDULE:

16. DISTRIBUTION STATEMENT (of this Report)

Unlimited

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17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

Unlimited

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18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Interferon gamma (IFNγ), interfons α+β (IFNα/β), augmented production, Listeria monocytogenes, macrophages, T cells, antiviral activity, purification.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Mice intravenously infected with an immunizing dose of the gram-positive bacterium, Listeria monocytogenes, produced circulating interferon (IFN) during the inductive phase of the anti-Listeria immune response. In addition to inducing IFN, the Listeria also dramatically altered the host's responsiveness to IFN inducing agents. Within 24 hours of infection, mice acquired a 50-fold greater than normal capacity to produce the alpha and/or beta IFN classes (IFNα/β) following intravenous injection of endotoxin. Serum IFN α/β levels peaked by 2 hours after which, high-levels of gamma IFN (IFNγ) were detected in the sera

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of Listeria-infected animals given the B cell mitogen. Similar studies carried out with the interferon inducing agent polyinosinic-polycytidylic acid (Poly(I)·Poly(C)) which, like endotoxin, induces peak levels of serum IFN 2 hours after intravenous injection, revealed that 24 hour infected mice produced only 4-8 times more IFN-α/β than non-infected mice. However, unlike endotoxin, Poly(I)·Poly(C) did not elicit IFN-γ synthesis in Listeria-infected animals.

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Annual Report

In addition to antiviral activity, the three antigenically distinct species (α,β,γ) of interferon (IFN) exhibit multiple biological activities, some of which suggest possible immunomodulatory roles for these molecules in either the generation and/or expression of immunity. The ultimate objective of our research is to determine the function of each IFN in cell-mediated immunity. The response of mice to Listeria monocytogenes offers an excellent model for studying the possible roles for IFNs in preimmunity resistance and acquired specific resistance to a falculitative intracellular pathogen. The expression of anti-Listeria resistance is dependent upon the generation of activated macrophages capable of expressing microbicidal function. Last year, we reported IFNγ is capable of activating macrophages, and earlier studies from this laboratory established a striking parallel between the development of T cell-mediated anti-Listeria immunity and an enhanced ability of spleen cells from responding mice to produce IFNγ in response to T cell polyclonal mitogens. Collectively, these findings strongly suggest a role for IFNγ in the expression of anti-Listeria resistance. During the past year, we have documented the IFN responses of mice during the course of an immunizing Listeria infection, and now are preparing to undertake studies which will examine the roles of each Listeria-induced IFN in the host's defense against bacterial infection.

1. IFN Responses of Listeria-Infected Mice

The serum IFN titers in endotoxin-injected and non-injected mice at progressive times following an immunizing dose of Listeria monocytogenes ($2x10^3$ viable organisms) are presented in Figure 1. Twenty-four hours after Listeria inoculation, low levels of IFN were detected in the serum. The Listeria-elicited serum IFN titers peaked (32 units) on the second day, and by the fifth day of infection, no IFN activity was detected. Listeria also dramatically enhanced the host's ability to produce IFN in response to the intravenous injection of endotoxin. Serum IFN levels measured 2 hours after endotoxin injection revealed that as early as 8 hours following Listeria inoculation, infected mice acquired the capacity to produce 5 times more endotoxin-induced IFN than non-infected animals. Maximum endotoxin-induced IFN titers occurred 24 hours following Listeria inoculation, with infected mice producing 64-fold more serum IFN activity than normal. Subsequent to this time, the enhanced responsiveness of the Listeria-infected mice to produce endotoxin-induced IFN slowly declined.

2. Temporal Appearance of Endotoxin-Induced Serum IFN

The kinetics of appearance for endotoxin-induced IFN in the sera of normal mice and mice inoculated with Listeria 24 hours earlier are presented in Figure 2. Maximum IFN levels were detected in the sera of both groups of animals 2 hours after the intravenous administration of 25μgs of endotoxin. At this time, the serum of the Listeria-infected mice possessed 32 times more IFN activity than serum of normal mice. These studies also revealed that endotoxin-induced IFN was detected earlier and the titers were significantly higher at all intervals in the sera of Listeria-infected mice than in normal animals. Two endotoxin-induced IFN responses were observed in the sera of normal mice. Following the initial serum IFN response, which was complete after 3 hours, a second minor peak of serum IFN activity was observed at 12 hrs. An examination of the descending portion of the serum IFN curve for the Listeria-infected mice revealed a slight upward inflection in the curve between 3-6 hrs. Evidence will be presented later showing this is due to a second endotoxin-induced IFN response.

3. Endotoxin and Poly(I)·Poly(C) Dose-Responses

Endotoxin-induced IFN dose-response studies carried out in 24 hour Listeria-infected and normal mice are presented in Figure 3, panel A. Graded concentrations of endotoxin were intravenously injected into groups of both animals. Two hours later, the mice were bled and the sera assayed for IFN activities. The dose-response curves established that mice infected one day earlier with an immunizing dose of Listeria, were at least 50 times more responsive to IFN induction by endotoxin than normal animals. Interferon levels plateaued in the infected animals treated with concentrations of endotoxin over 1μg, where the titers of IFN were at least 50-fold greater than that of normal mice given the highest concentration of endotoxin (100μg) tested.

Since the synthetic polyribonucleotide Poly(I)·Poly(C) also induces peak serum IFN levels 2 hours after intravenous injection, it was deemed of interest to compare the relative responsiveness of Listeria-infected mice to Poly(I)·Poly(C) IFN induction in a dose-response study (Fig. 3, panel B). The Poly(I)·Poly(C) dose-response curves for IFN induction in normal and 24 hour Listeria-infected mice exhibit similar slopes. Moreover, although the Listeria-infected animals proved more responsive to Poly(I)·Poly(C), the yields of IFN elicited by all concentrations were only 4-8 fold higher than those produced by similarly treated normal mice.

4. Endotoxin-Induced IFN in Spleen Cultures Derived from Listeria-Infected Mice

The spleen is a source of endotoxin-induced serum IFN (3,4,14). Therefore, studies were carried out to determine the responsiveness of spleen cells obtained from Listeria-infected mice to produce IFN after endotoxin stimulation. Spleen cell cultures were established from normal mice and from mice either inoculated 1 or 6 days earlier with Listeria. The cultures were incubated with endotoxin (5μgs/ml) and at the designated times, portions of the media were collected and assayed for IFN activity (Fig. 4). Spleen cells obtained from mice infected 1 day earlier with Listeria, produced the highest levels of endotoxin-induced IFN activity. These cultures also produced IFN earlier (2hrs) than either of the two other experimental groups of spleen cells. The endotoxin-induced spleen cultures of both normal and 6 day-infected mice did not secrete IFN until after 2 hours. However, like cultures from 1 day-infected mice, peak levels of IFN in normal cultures were attained by 6 hours, whereas cultures from 6 day-infected mice continued producing IFN after this time.

5. The Requirement of Viable Listeria for Augmented IFN Induction

The foregoing studies established that an immunizing dose of viable Listeria could elicit IFN, as well as enhance the responsiveness of the infected mice to IFN inducing agents. In Table 1, are presented the findings of studies designed to determine if by increasing the number of inoculated organisms would further enhance the yields of endotoxin-induced IFN, and whether viable Listeria are required for the enhanced endotoxin-induced IFN response. Increasing the Listeria inoculum 100 times over the usual immunizing inoculum resulted in only a marginal 2-fold increase in serum IFN activity in mice receiving endotoxin 2 hrs earlier. Listeria rendered non-viable by either heat or ultraviolet irradiation were incapable of invoking the augmented endotoxin-induced IFN response observed in animals treated with the same number of viable organisms.

6. T-cell Independence for Enhanced 2 Hour Endotoxin-Induced Serum IFN in Listeria-Infected Mice

Thymectomized, irradiated, and bone marrow reconstituted (TXB) $AB6F_1$ mice or congenitally athymic (nu/nu) mice of BALB/c background were infected with $2x10^5$ viable Listeria and 24 hours later injected intravenously with endotoxin. Two hours later, the sera from the Listeria -infected and similar groups of non-infected mice were collected and assayed for IFN (Table 2). The infected TXB $AB6F_1$ and athymic nude mice produced significantly higher yields of serum IFN 2 hours after endotoxin induction than their respective non-infected control groups. However, the differences in magnitude of the IFN responses between non-infected and infected T cell-deficient groups were not as great (~50x) as those previously observed between infected and normal $AB6F_1$ immunocompetent mice. This was due to the fact that the non-infected T cell-deficient mice produced relatively more endotoxin-induced IFN than non-infected $AB6F_1$ immunocompetent mice.

7. Interferon Responses to Different Agents at Progressive Times Post Listeria Inoculation

The IFN responses of mice to three agents were determined at times prior to, after, and at the peak of the anti-Listeria T cell-mediated immune response on day 6. Two and 6 hours following the intravenous injection of endotoxin, Poly(I)·Poly(C), or 10^6 viable Listeria, the various groups of mice were bled and the sera assayed for IFN (Table 3). Peak 2 hour endotoxin-induced IFN levels occurred 1 day following an immunizing Listeria dose, after which, the early 2 hour IFN response gradually declined by day 15 to a level still 10 times higher than normal. In contrast to the peak 2 hour endotoxin-induced IFN response, which occurred 1 day following Listeria inoculation, the maximum 6 hour serum IFN response elicited by endotoxin occurred on day 6. On the sixth day, the 6 hour serum IFN titer was equivalent to the earlier 2 hour serum titer, which was only 4-fold lower than the peak 2 hour response observed in mice 1 day after Listeria inoculation. However, the 6 hour serum IFN levels produced by mice injected with Listeria 15 days earlier, were greatly diminished relative to the 2 hour IFN titers detected in these mice. The 2 and 6 hour Poly(I)·Poly(C)-induced serum IFN levels were elevated only on day 1 of Listeria infection. The highest 2 and 6 hour serum IFN titers induced by injection of 10^5 viable Listeria, coincided with the peak of anti-Listeria T cell-mediated immunity on the sixth day after injection of an immunizing Listeria dose.

8. Antigenic Analysis of Endotoxin-Induced and Poly(I)·Poly(C)-Induced Serum IFNs

The availability of anti-murine IFNα/β neutralizing serum and monoclonal anti-murine IFNγ neutralizing antibody, enabled the qualitative and quantitative analysis of the IFNα/β and IFNγ activities present in endotoxin-induced or Poly(I)·Poly(C)-induced serum IFN samples (Table 3). It should be stated that as in the human IFN system, the murine IFNα and IFNβ molecules are antigenically distinct not only from one another, but also from IFNγ. Specific anti-murine IFNα and IFNβ neutralizing antibodies are not available, however, anti-IFNα/β is available. Antigenic analyses of the IFN classes (total IFNα/β or IFNγ) existing in the various endotoxin-induced or Poly(I)·Poly(C)-induced serum IFN samples (Table 3), were performed by reacting each sample with an excess (>500 neutralizing units) of anti-IFNα/β or anti-IFNγ antibody, as well as with a mixture of both antibodies. The reacted samples were then assayed for non-neutralized antiviral activities. The results of these studies revealed that the Poly(I)·Poly(C)-induced antiviral activities in all sera tested were mediated exclusively by IFNα/β (Table 4). Likewise, IFNα/β mediated the antiviral activity in the 2 hour sera of endotoxin-induced mice. However, the later

appearing 6 hour endotoxin-induced serum IFN molecules produced by mice infected 1 and 6 days earlier with Listeria, were mixtures of IFNα/β and IFNγ . Based on the antiviral activity remaining after the antibody treatments, IFNγ is the major IFN species in these 6 hour sera.

These studies establish that shortly after an immunizing dose of Listeria, mice acquire a greatly augmented capaci to produce endotoxin-induced IFNα/β and following the peak IFNα/β response, IFNγ is produced. The capacity of Listeria-infected mice to produce elevated IFNα/β titers in response to endotoxin is long lived and diminishes slowly, as evidenced by the significantly higher IFNα/β titers produced by mice infected 15 days earlier, as compared to normal mice. The capacity of Listeria-infected mice to produce IFNγ following induction with the B cell mitogen is more transitory, peaking along with anti-Listeria immunity on day 6, and is lost 15 days after inoculation with Listeria.

Figure 1

Listeria-induced and endotoxin-induced serum interferon levels during infection. Listeria-induced IFN serum levels (□--) and serum IFN levels measured 2 hours post intravenous injection of 25 μgs of endotoxin (○—) in mice at progressive time following inoculation of an immunizing Listeria dose.

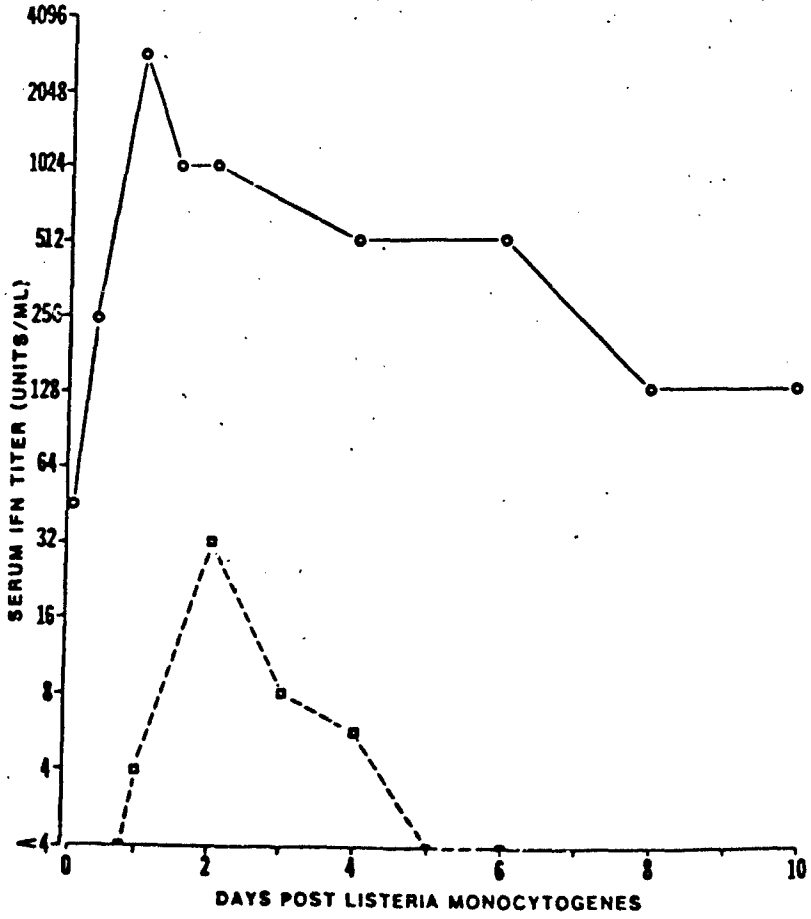

Figure 2

Kinetics of IFN appearance in the sera of normal and 1 day Listeria-infected mice. Groups of normal and 1 day Listeria-infected mice were injected intravenously with 25 μgs of endotoxin (o hr) and bled at the indicated times.

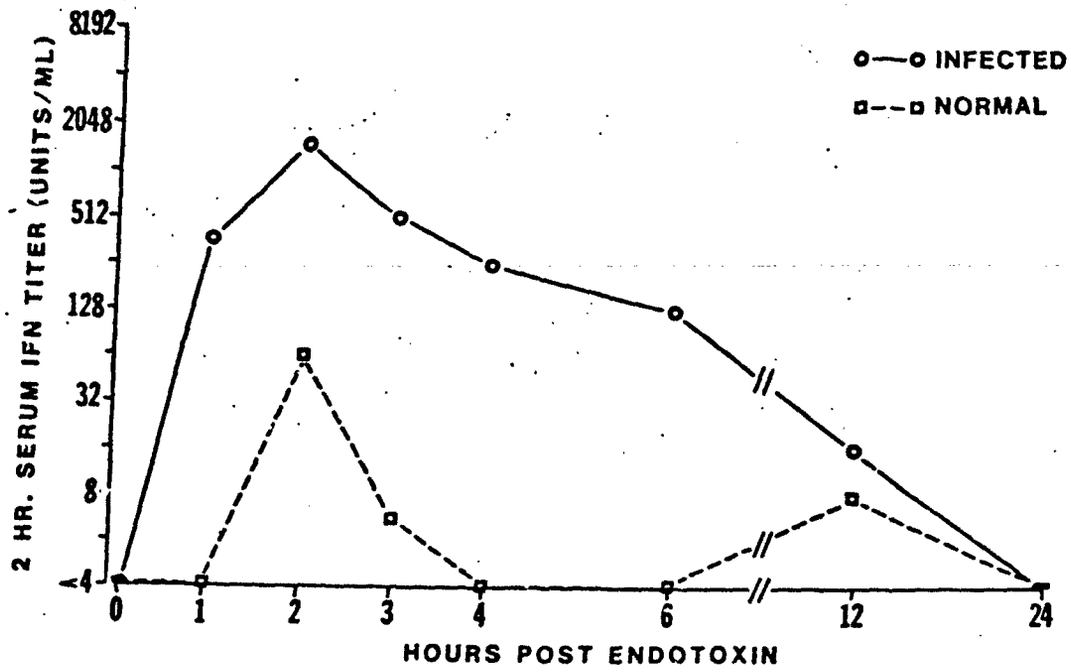

Figure 3

Endotoxin (Panel A) and Poly(I)·Poly(C) (Panel B) induced serum IFN levels in normal and 1 day Listeria-infected mice. Groups of 5 mice were injected intravenously with the designated concentrations of either IFN inducing agent and bled 2 hours later.

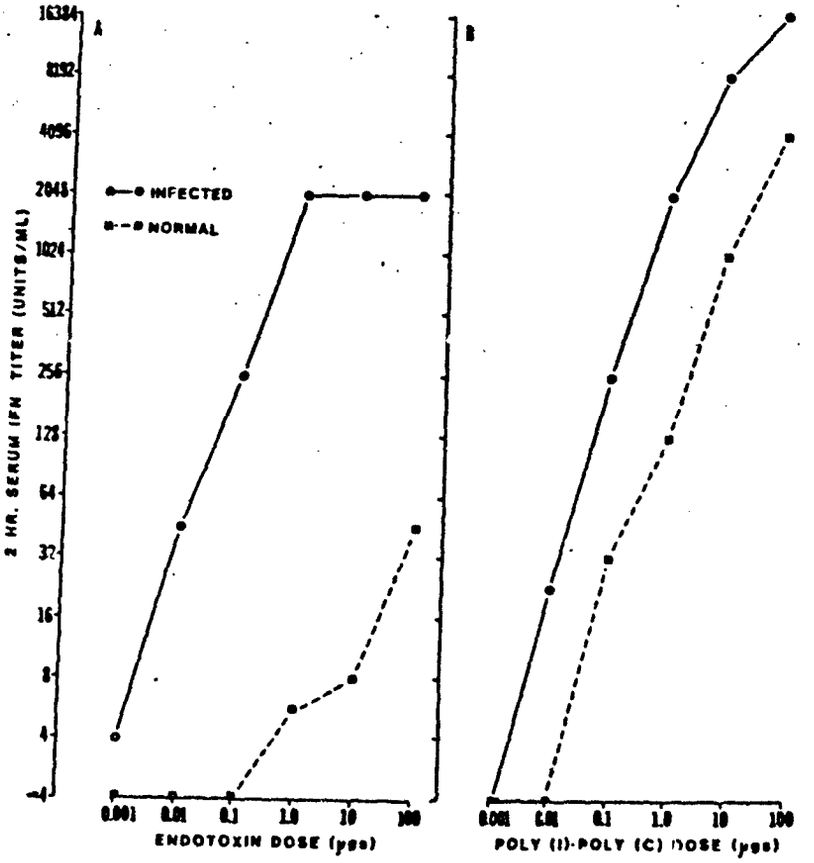

Figure 4

Endotoxin-induced IFN synthesis in spleen cell cultures derived from normal, 1 and 6 day Listeria-infected mice. Replicate spleen cell cultures (10^7 cells/ml) were incubated with 5 µgs/ml of endotoxin at 0 hr and samples of medium collected at the designated times and assayed for IFN activity.

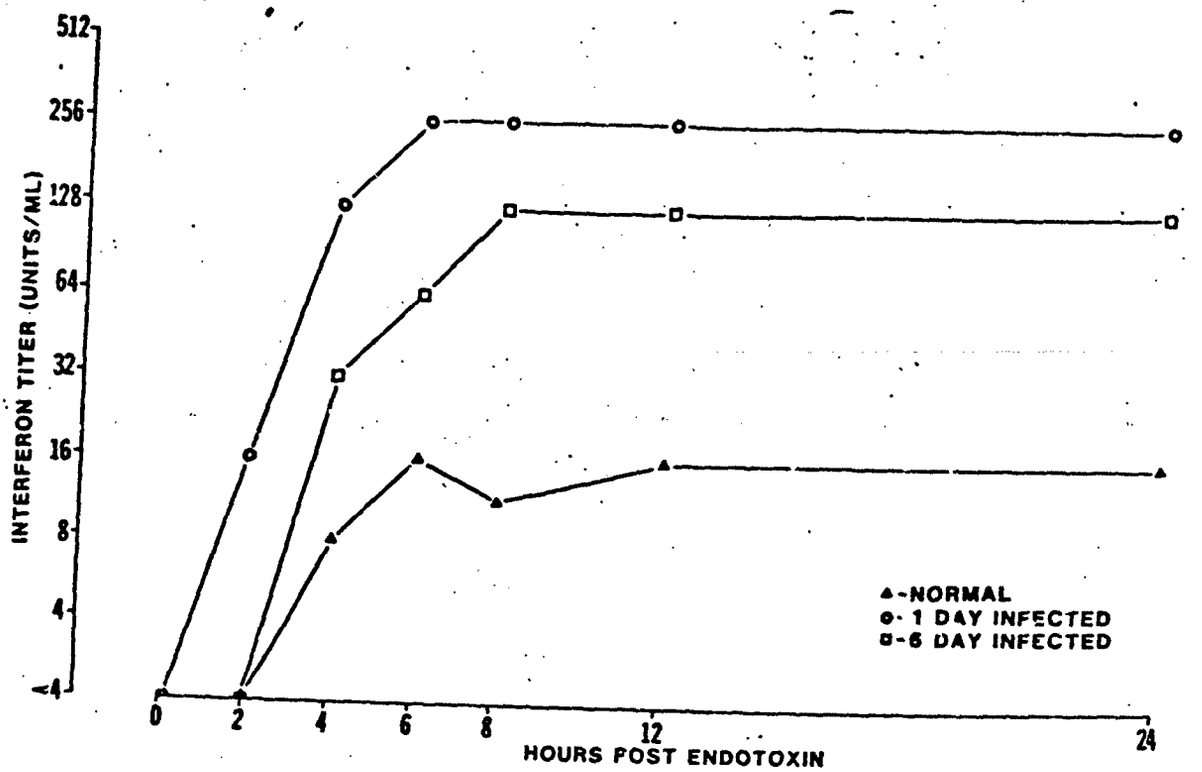

Table 1

Viable Listeria are Required for Enhanced Endotoxin-Induced Interferon Responses

Treatment 24h prior to endotoxin	Serum inteferon titer(units/ml) 2h post endotoxin[c]
None	48
$2x10^3$ viable Listeria, i.v.	2048
$2x10^5$ viable Listeria, i.v.	4096
$2x10^5$ killed[a] Listeria, i.v.	32
$2x10^5$ U.V. killed[b] Listeria, i.v.	32

[a]Broth culture of Listeria incubated at 60°C/1hr. Following such treatment, no viable Listeria were detected by plating on tryptic case soy agar.

[b]Details of U.V. irradiation procedure presented in Materials and Methods.

[c]Intravenous injection of 25 µgs of endotoxin.

Table 2

Enhanced Responsiveness to Endotoxin Interfero. Induction in TXB and Athymic Nude Mice Following Listeria Infection

Mouse type	Treatment 24h prior to endotoxin	Serum interferon(units/ml) 2h post endotoxin[c]
TXB[a]	none	384
"	$2x10^5$ Listeria, i.v.	2048
Athymic nude	none	192
"	$2x10^5$ Listeria, i.v.	6144

[a] TXB: thymectomized, irradiated, and bone marrow reconstituted $AB6F_1$ mice.

[b] Congenitally athymic nude (nu/nu) mice of BALB/c background.

[c] 25 µgs endotoxin injected intravenously.

Table 3

Endotoxin and Poly(I)·Poly(C)-Induced Serum Interferon Levels at Various Days Post Listeria Infection

Day of Infection	Inducing agent[a]	Serum interferon titers (units/ml) at 2 h	6 h
non-infected	Endotoxin	32	6
	Poly(I)·Poly(C)	2048	192
	Listeria	<4	<4
1 day	Endotoxin	2048	192
	Poly(I)·Poly(C)	8192	2048
	Listeria	4	16
6 day	Endotoxin	512	512
	Poly(I)·Poly(C)	2048	64
	Listeria	32	64
15 day	Endotoxin	384	32
	Poly(I)·Poly(C)	3072	256
	Listeria	<4	6

[a]At 0 h groups of mice were injected with 5 µgs of endotoxin, 25 µgs Poly(I)·Poly(C) or 10^6 viable *Listeria*.

Table 4

Antigenicities of 2 and 6 hour Endotoxin and Poly(I)·Poly(C)-Induced Serum Interferons Produced by Listeria-Infected Mice

Day of infection	IFN inducing agent[a] at 0 h	Hour of serum IFN sample	Antiviral activities (units/ml)[b] after reaction with excess			
			none	anti-α/β	anti-γ	anti-α/β+anti-γ
non-infected	Endotoxin	2	64	<8	64	<8
	Poly(I)·Poly(C)	2	512	<8	512	<8
	Poly( )·Poly(C)	6	256	<8	128	<8
1	Endotoxin	2	512	<8	256	<8
	Poly(I)·Poly(C)	2	128	<8	256	<8
	Endotoxin	6	256	256	96	<8
	Poly(I)·Poly(C)	6	256	<8	256	<8
6	Endotoxin	2	256	<8	128	<8
	Poly(I)·Poly(C)	2	256	<8	128	<8
	Endotoxin	6	256	128	32	<8
	Poly(I)·Poly(C)	6	256	<8	256	<8
15	Endotoxin	2	512	<8	512	<8
	Poly(I)·Poly(C)	2	512	<8	512	<8
	Endotoxin	6	32	<8	32	<8
	Poly(I)·Poly(C)	6	128	<8	256	<8

[a] Endotoxin (5 µgs) or Poly(I)·Poly(C) (25 µgs) injected intravenously at 0 h.

[b] Sera Originally with titers >512 (Table 2) were diluted to ~512 units/ml.