INFECTION AND IMMUNITY, Dec. 1985, p. 877–880
0019-9567/85/120877-04$02.00/0

Vol. 50, No. 3

Induction of Alpha/Beta Interferon and Gamma Interferon in Mice Infected with *Listeria monocytogenes* during Pregnancy

AKIO NAKANE,* TOMONORI MINAGAWA, AND IZUMI YASUDA

Department of Microbiology, Hokkaido University School of Medicine, Kita 15, Nishi 7, Kita-Ku, Sapporo 060, Japan

Received 22 July 1985/Accepted 30 August 1985

Alpha/beta interferon (IFN-α/β) was induced in the bloodstream of mice 48 h after intravenous infection with *Listeria monocytogenes*, whereas IFN-γ was induced in the bloodstream 6 h after stimulation with specific antigen on day 5 of infection in virgin mice. In contrast, no IFN-α/β or IFN-γ was produced in the bloodstream of pregnant mice after *L. monocytogenes* infection. However, unusual acid-labile IFN-α/β instead of IFN-γ was produced in some of the pregnant mice in response to specific antigen. The bacterial growth in the organs of pregnant mice in the early stage of infection was normal, but resulted in the delay of T-cell-dependent elimination of bacteria from the organs of pregnant animals in the late stage, and numerous bacteria were detected in both the placenta and the fetus. The significance of the IFN system induced by *L. monocytogenes* infection in pregnant mice is discussed.

Listeria monocytogenes, a facultative intracellular-growing pathogen, gives rise to disease in various forms in humans and in a wide range of other animals (24). The immunosuppressed individuals, including patients with neoplastic diseases or those treated with immunosuppressive therapies, are particularly susceptible to *L. monocytogenes* infection. Furthermore, *L. monocytogenes* causes maternal and fetal infections during pregnancy in both humans and other animals (1). There is evidence that cell-mediated immunity is suppressed during pregnancy (26) and that pregnancy diminishes the ability of the host to resist infection by several other pathogens (5, 6, 9) as well as by *L. monocytogenes* (13).

Elimination of *L. monocytogenes* from the tissues of infected animals is performed by two steps involving T-cell-independent mechanisms in the early phase and T-cell-dependent mechanisms in the late phase of infection (14–16, 21). Our recent study (20) demonstrated that either alpha/beta interferon (IFN-α/β) or IFN-γ was produced in the bloodstream of mice depending on the immunological status of the host during infection with *L. monocytogenes*. IFN-α/β would be produced in the early phase of primary infection with the bacterium, whereas, IFN-γ would be induced by stimulation with specific antigen or by reinfection with *L. monocytogenes* in mice after the specific immunity had been established.

Because of these findings, we considered it of great interest to determine the ability of mice to produce IFN-α/β and IFN-γ as one of parameters of the immune status during pregnancy in *L. monocytogenes*-infected mice. Our present studies show that the production of both IFN-α/β and IFN-γ in *L. monocytogenes*-infected mice during pregnancy is markedly suppressed.

MATERIALS AND METHODS

Mice. Male and female mice of the ddY strain (obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu, Shizuoka, Japan), 10 to 16 weeks old, were used. The onset of pregnancy was ascertained by mating mice for a maximum of 4 days and examining them for the presence of a postcoital plug after day 2. Pregnant mice were used for experiments at 15 to 18 days of gestation and were age matched with virgin mice.

Bacteria and bacterial antigen. *L. monocytogenes* 1b 1684 cells, kindly provided by T. Nagai, Department of Microbiology, Sapporo Medical College Hospital, Sappora, Japan, were prepared as previously reported (17). The concentration of washed cells was adjusted spectrophotometrically at 550 nm. Mice were infected intravenously with 0.2 ml of a solution containing 10^4 CFU of viable *L. monocytogenes* cells in 0.01 M phosphate-buffered saline (PBS; pH 7.4). *Listeria* cell wall fraction (LCWF) was prepared as previously reported (20). Mice were injected intravenously with 50 μg of LCWF suspended in PBS on day 5 of infection with *L. monocytogenes*.

Assays and characterization of IFN. IFN activities in specimens of serum, placenta, fetus, and amniotic fluid obtained from an individual mouse were measured. Extracts of the placenta and fetus were prepared as follows: These reproductive tissues were dissected and washed twice with PBS. A 20% (wt/vol) homogenate of each specimen was then prepared in PBS and clarified by centrifugation for 10 min at 2,000 × *g* and then for 30 min at 12,000 × *g*. The extracts obtained were sterilized by filtration through a membrane filter (pore size, 0.45 nm; Millipore Corp., Bedford, Mass.) and stocked at −70°C until the IFN assay. The IFN assay was carried out by the dye binding method (2) with L-929 cells and vesicular stomatitis virus (Indiana strain) as previously reported (19). When sera, placental extracts, fetal extracts, and amniotic fluid were assayed, cell monolayers in each well of a 96-well microplates (Nunc, Roskilde, Denmark) were washed twice with RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 4% fetal calf serum (GIBCO) used for assays to avoid the effect of a nonspecific virus inhibitor(s) in the samples (22). Neutralization tests with anti-mouse IFN-α/β antibody (NIAID catalog no. G-024-501-568), kindly provided by G. J. Galasso, Microbiology and Infectious Disease Program, National Institute of Allergy and Infectious Diseases, and acid stability tests of IFN samples were carried out as reported previously (16).

Determination of viable *L. monocytogenes* cells in the organs. The numbers of viable *L. monocytogenes* cells in spleens, livers, placentas, and fetuses of the infected animals

* Corresponding author.

TABLE 1. IFN production in the blood of virgin and pregnant mice by primary infection with *L. monocytogenes*

Type of mouse[a]	Specimen[b]	No. of mice	IFN titer (IU/ml) after:		
			No treatment	Anti-IFN-α/β[c] treatment	Treatment at pH 2.0[d]
Virgin	Serum	20	66 ± 34	<4	59 ± 34
Pregnant	Serum	10	<4	ND[e]	ND
	Placenta	10	<4	ND	ND
	Fetus	10	<4	ND	ND
	Amniotic fluid	10	<4	ND	ND

[a] Virgin and pregnant (15 days of gestation) mice were infected intravenously with 10^4 CFU of *L. monocytogenes*.
[b] Specimens were taken on day 2 of infection.
[c] The neutralization test was carried out as described in the text.
[d] Samples were dialyzed against 0.2 M KCl–HCl buffer (pH 2.0) at 4°C for 48 h, followed by dialysis against minimal essential medium at 4°C for 24 h.
[e] ND, Not done.

were established by plating serial 10-fold dilutions of organ homogenate in PBS on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.). Colony counts were routinely performed 18 to 24 h later.

RESULTS

IFN production in the bloodstream of mice by primary infection with *L. monocytogenes*. After virgin and pregnant mice were infected intravenously with 10^4 CFU of *L. monocytogenes*, the antiviral activity in the sera obtained from them on day 2 of infection was determined (Table 1). Although acid-stable IFN-α/β was demonstrated in all the virgin mice, no antiviral activity was detected in the pregnant mice. Similarly, the placental extract, the fetal extract, and the amniotic fluid from all the pregnant mice showed no antiviral activity.

IFN production in the bloodstream of *L. monocytogenes*-infected mice stimulated with the specific antigen. LCWF (50 μg) was injected intravenously into mice on day 5 of the *L. monocytogenes* infection, and IFN activity in the blood of each mouse was determined 6 h later (Table 2). IFN was produced in the bloodstream of all the virgin mice (32 to 256 IU/ml). In contrast, IFN activity was detected in the sera of only 4 of 19 pregnant mice, and their IFN titers were markedly low (8 to 16 IU/ml). No antiviral activity was demonstrated in placental or fetal extracts, even in mice for which IFN was detected in the serum specimens.

IFN induced by specific antigen in the bloodstream of pregnant mice was characterized (Table 3). The IFN activity in the sera of virgin mice was not neutralized by anti-mouse IFN-α/β antibody and was inactivated by acid treatment.

TABLE 2. IFN production in response to specific antigen in the blood of pregnant mice infected with *L. monocytogenes*

Type of mouse	No. of mice	IFN titer (IU/ml) in samples of[a]:		
		Serum	Placenta	Fetus
Virgin	30	148 ± 82	—[b]	—
Pregnant	4	12 ± 4	<4	<4
Pregnant	19	<4	<4	<4

[a] After virgin and pregnant (15 days of gestation) mice were infected with *L. monocytogenes*, LCWF (50 μg) was injected intravenously into the mice on day 5 of infection. IFN activities in sera, placental extracts, and fetal extracts were determined 6 h later.
[b] —, Not done.

One of four serum specimens showed the same characteristics as did both IFN produced in virgin mice and standard IFN-γ. On the contrary, the other three samples were neutralized with anti-mouse IFN-α/β, antibody in the same way as standard IFN-α/β, but were acid labile in the same way as IFN-γ.

Bacterial growth in the organs of pregnant mice. The number of *L. monocytogenes* cells in spleens, livers, placentas, and fetuses was estimated on day 2 of infection, when IFN-α/β governed the host, and on day 5, when IFN-γ governed it after *L. monocytogenes* infection (Table 4). The number of *L. monocytogenes* cells in spleens and livers was not different in virgin and pregnant mice on day 2 of infection. However, the efficiency of the elimination of bacteria decreased in pregnant mice on day 5 of infection, while antigen-specific elimination of bacteria processed in virgin mice. Although the number of *L. monocytogenes* cells in placentas and fetuses was below the detectable level on day 2 of infection, numerous bacteria were demonstrated in those organs on day 5 of infection.

DISCUSSION

It was demonstrated that the *L. monocytogenes*-induced production of both IFN-α/β and IFN-γ in the bloodstream of pregnant mice was suppressed. Our previous paper showed that suppression of IFN-γ production resulted in the delay of T-cell-dependent elimination of bacteria from the organs (20). In fact, in pregnant mice numerous bacteria were observed to invade the placentas and fetuses in addition to the spleens and livers. There are two possible mechanisms to explain the suppression of IFN-γ production in pregnant mice; IFN-α/β-dependent and IFN-α/β-independent suppression. It is known that cell-mediated immunity is impaired during pregnancy. Although the mechanism of impairment has not been clarified, the presence of various suppressive agents involving nonspecific suppressor cells, steroid hormones, α-fetoprotein, α_2-glycoprotein, and immunoglobulin G-blocking antibody, has been documented (26). It is possible that the production of IFN-γ, a representative lymphokine, may be impaired by these suppressive agents generated during pregnancy. In addition to such IFN-α/β-independent suppression, an IFN-α/β-dependent mechanism might be possible. In our previous study (20), it was shown that IFN-α/β produced in the early stage of *L. monocytogenes* infection might play a key role as a messenger to generate antigen-specific T cells involving IFN-γ production and acquired resistance to the infection. To do

TABLE 3. Characterization of IFN induced by specific antigen in the blood of pregnant mice infected with *L. monocytogenes*

Source of serum and IFN	No. of samples	IFN titer (IU/ml) after:		
		No treatment	Anti-IFN-α/β[a] treatment	Treatment at pH 2.0[b]
Virgin mice	2, 5, 8, 17, 25	64	64	<4
Pregnant mice	1	16	<4	<4
Pregnant mice	4	8	<4	<4
Pregnant mice	16	8	<4	<4
Pregnant mice	22	16	16	<4
NDV-induced IFN-α/β[c]		360	<16	360
Specific antigen-induced IFN-γ in BCG-sensitized mice[c]		1,360	1,320	<10

[a] See Table 1, footnote *a*.
[b] See Table 1, footnote *d*.
[c] Serum IFN-α/β and IFN-γ were prepared as previously reported (15).

this, IFN-α/β was induced in the circulation of mice infected intravenously with *L. monocytogenes* 24 to 72 h after infection, and IFN-α/β could be produced by mainly asialo GM1-bearing cells, which are equivalent to natural killer cells (12). After 5 days of infection when the specific resistance against reinfection with *L. monocytogenes* was established, IFN-γ could be induced in the bloodstream 3 to 6 h after stimulation with specific antigen. However, IFN-γ production was suppressed when IFN-α/β production had been inhibited by treatment with anti-asialo GM1 antibody or when the IFN produced had been neutralized with anti-mouse IFN-α/β antibody. Also, the specific resistance against reinfection with *L. monocytogenes* was suppressed in IFN-α/β-depleted mice. On the other hand, no significant effect on either IFN-γ production or specific resistance was observed when these antibodies had been administered after IFN-α/β production. Therefore, IFN-α/β must be essential for the generation of antigen-specific T cells during *L. monocytogenes* infection. Additionally, natural killer cells may play an important role in the generation of antigen-specific T cells as accessory cells as well as IFN-α/β-producing cells. Recently, the significance of natural killer cells as accessory cells was demonstrated (25). The generation of alloimmune cytotoxic T cells was suppressed in asialo GM1 antibody-pretreated mice, and the generation was restored by IFN or interleukin 2. It was also observed that administration of anti-IFN-α/β antibody (8) or anti-asialo GM1 antibody (10) resulted in impairment of resistance against herpes simplex virus type 1 infection in mice. On the basis of these findings, it is possible that dysfunction of natural killer cells may cause impairment of IFN-α/β production in pregnant mice during the early stage of *L. monocytogenes* infection and that *Listeria*-specific T cells, which can produce IFN-γ, may not be fully generated.

The bloodstreams of some of the pregnant mice contained unusual acid-labile IFN-α/β after stimulation with specific antigen on day 5 of *L. monocytogenes* infection. Unusual acid-labile IFN-α can be detected in the sera obtained from patients with systemic lupus erythematosus (11, 23) or with acquired immune deficiency syndrome (4). A common characteristic among these diseases and pregnancy is the defective functions of the immunoregulatory T-cell circuits (3, 7, 26). We noticed the production of unusual acid-labile IFN-α/β in response to specific antigen in *Mycobacterium bovis* BCG-sensitized mice (18). After induction of IFN-γ by specific antigen in BCG-sensitized mice, the mice developed a hyporeactive state against IFN-γ production, but acid-labile IFN-α/β instead of IFN-γ was produced in their bloodstreams. We assume that a mechanism of production of acid-labile IFN-α/β may be related to that of IFN-γ rather than to that of normal acid-stable IFN-α/β. To prove our hypothesis, more detailed studies to elucidate the producing mechanism and the significance of acid-labile IFN-α/β are necessary and are currently been carrying out.

TABLE 4. Number of *L. monocytogenes* cells in the organs of virgin and pregnant mice

Type of mouse	Organ	Log bacteria/organ[a] on:	
		Day 2	Day 5
Virgin	Spleen	5.80 ± 0.40	4.39 ± 0.19
	Liver	5.20 ± 0.33	4.55 ± 0.27
Pregnant	Spleen	5.33 ± 0.05	6.12 ± 0.11
	Liver	4.11 ± 0.19	6.86 ± 0.76
	Placenta	<3.00	8.57 ± 0.40
	Fetus	<3.00	7.05 ± 1.03

[a] The number of viable *L. monocytogenes* cells in the organs of infected animals was established by plating out 10-fold dilutions of the organ homogenates in PBS on Trypticase soy agar.

ACKNOWLEDGMENTS

We thank H. Iida for advice and encouragement and N. Suzuki-Nakane for technical assistance.

This study was supported in part by a grant from the Ministry of Education, Science, and Culture, Japan.

LITERATURE CITED

1. **Andriole, V. T.** 1982. Medical complications during pregnancy, p. 302–332. *In* G. N. Burrow, and T. F. Ferris (ed.), Bacterial infection. The W. B. Saunders Co., Philadelphia.
2. **Armstrong, J. A.** 1971. Semi-micro, dye-binding assay for rabbit interferon. Appl. Microbiol. **21**:723–725.
3. **Delfraissy, J. F., P. Segond, P. Galanaud, C. Wallon, P. Massias, and J. Dormonti.** 1980. Suppressed primary in vitro antibody responses in untreated systemic lupus erythemotosus. Helper cell defect and lack of defective suppressor cell function. J. Clin. Invest. **66**:141–148.
4. **DeStefano, E., R. M. Friedman, A. E. Friedman-Kien, J. J. Goedert, D. Henriksen, O. T. Preble, J. A. Sonnabend, and J. Vilcěk.** 1982. Acid-labile human leukocyte interferon in homo-

sexual men with Kaposi's sarcoma and lymphadenopathy. J. Infect. Dis. **146**:452–455.
5. **Drutz, D. J., and M. Huppert.** 1983. Coccidioidomycosis: factors affecting the host-parasite interaction. J. Infect. Dis. **147**:469–474.
6. **Farber, P. A., and L. A. Glasgow.** 1968. Factors modifying host resistance to virus infection. II. Enhanced susceptibility of mice to encephalomyocarditis virus infection during pregnancy. Am. J. Pathol. **53**:463–481.
7. **Gottlieb, M. S., R. Schroff, H. M. Schanker, J. P. Weisman, P. I. Fan, R. A. Wolf, and A. Saxon.** 1981. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N. Engl. J. Med. **305**:1425–1431.
8. **Gesser, I., M. G. Tovey, C. Maury, and M. T. Bandu.** 1976. Role of interferon in the pathogenesis of virus diseases in mice as demonstrated by the use of anti-interferon serum. II. Studies with herpes simplex, moloney sarcoma, vesicular stomatitis, Newcastle disease, and influenza virus. J. Exp. Med. **144**:1316–1323.
9. **Griffith, B. P., H. L. Lucia, J. L. Tillbrook, and G. P. Hsiung.** 1983. Enhancement of cytomegalovirus infection during pregnancy in guinea pig. J. Infect. Dis. **147**:990–998.
10. **Habu, S., K. Akamatsu, N. Tamaoki, and K. Okumura.** 1984. *In vivo* significance of NK cells on resistance against virus (HSV-1) infections in mice. J. Immunol. **133**:2743–2747.
11. **Hooks, J. J., G. W. Jordan, T. Cupps, H. M. Moutsopoulos, A. S. Fauci, and A. L. Notkins.** 1982. Multiple interferons in the circulation of patients with systemic lupus erythematosus and vasculitis. Arthritis Rheum. **25**:396–400.
12. **Kasai, M., M. Iwamori, K. Nagai, K. Okumura, and T. Tada.** 1980. A glycolipid on the surface of mouse natural killer cells. Eur. J. Immunol. **10**:175–180.
13. **Luft, B. J., and J. S. Remington.** 1982. Effect of pregnancy on resistance to *Listeria monocytogenes* and *Toxoplasma gondii* infections in mice. Infect. Immun. **38**:1164–1171.
14. **Mackaness, G. B.** 1962. Cellular resistance to infection. J. Exp. Med. **116**:381–402.
15. **Mackaness, G. B.** 1969. The influence of immunologically committed lymphoid cells on macrophage activity in vivo. J. Exp. Med. **129**:973–992.
16. **Mitsuyama, M., K. Takeya, K. Nomoto, and S. Shimotori.** 1978. Three phases of phagocyte contribution to resistance against *Listeria monocytogenes*. J. Gen. Microbiol. **106**:165–171.
17. **Nakane, A. and T. Minagawa.** 1981. Alternative induction of IFN-α and IFN-γ by *Listeria monocytogenes* in human peripheral blood mononuclear leukocyte cultures. J. Immunol. **126**:2139–2142.
18. **Nakane, A., and T. Minagawa.** 1982. Induction of alpha and beta interferons during the hyporeactive state of gamma interferon by *Mycobacterium bovis* BCG cell wall fraction in *Mycobacterium bovis* BCG-sensitized mice. Infect. Immun. **36**:966–970.
19. **Nakane, A., and T. Minagawa.** 1983. Alternative induction of alpha/beta interferons and gamma interferon by *Listeria monocytogenes* in mouse spleen cell cultures. Cell. Immunol. **75**:283–291.
20. **Nakane, A., and T. Minagawa.** 1984. The significance of alpha/beta interferons and gamma interferon produced in mice infected with *Listeria monocytogenes*. Cell. Immunol. **88**:29–40.
21. **North, R. J.** 1973. Cellular mediators of anti-*Listeria* immunity as an enlarged population of short-lived, replicating T cells. Kinetics of their production. J. Exp. Med. **138**:342–355.
22. **Ohno, S., F. Kato, H. Matsuda, N. Fujii, and T. Minagawa.** 1982. Detection of gamma interferon in the sera of patients with Behçet's disease. Infect. Immun. **36**:202–208.
23. **Preble, O. T., R. J. Black, R. M. Friedman, J. H. Klippel, and J. Vilček.** 1982. Systemic lupus erythematosus: presence in human serum of unusual acid-labile leukocyte interferon. Science **216**:429–431.
24. **Smith, G., and G. Wilson.** 1984. *Erysipelothrix* and *listeria* infections, p. 23–31. *In* G. Wilson, A. Miles, and M. T. Packer (ed.), Topley and Wilson's principles of bacteriology, virology and immunity, vol. 3, bacterial diseases, 7th ed. Edward Arnold, London.
25. **Suzuki, R., S. Suzuki, N. Ebina, and K. Kumagai.** 1985. Suppression of alloimmune cytotoxic T lymphocyte (CTL) generation by depletion of NK cells and restoration by interferon and/or interleukin 2. J. Immunol. **134**:2139–2148.
26. **Weinberg, E. D.** 1984. Pregnancy-associated depression of cell-mediated immunity. Rev. Infect. Dis. **6**:814–831.