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Male sterility of transgenic mice carrying exogenous mouse interferon-β gene under the control of the metallothionein enhancer-promoter

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As an approach to elucidating the roles of interferon (IFN) in the normal physiology and diseases of animals, transgenic mice carrying extra mouse IFN-β genes under the control of a mouse metallothionein I enhancer-promoter were constructed. Upon induction with Cd^{2+}, IFN activity (15-430 IU/ml) was detected in the sera of six out of ten transgenic mouse lines so far obtained. Synthesis of mRNA of the transgene was observed in the liver, the testis and less abundantly in the brain. Interestingly, IFN mRNA was constitutively synthesized in the testis where substantial levels of IFN accumulated without heavy metal induction, whereas synthesis in the liver was mostly dependent on induction by Cd^{2+}. Since IFN activity in the serum also depended on heavy metal induction, the IFN in the serum may be produced mainly in the liver. All males expressing the IFN gene in the testis were found to be sterile. Testes were involuted and contained few mature sperm, and degeneration of spermatocytes and spermatids was observed. These findings suggest that high levels of IFN are harmful to spermatogenesis and can cause male sterility.

Key words: interferon/metallothionein I-enhancer/spermatogenesis/transgenic mouse

Introduction

In addition to its antiviral activity, it is well known that interferon (IFN) exerts various biological effects on cells, including inhibition of cell growth (Iwakura *et al.*, 1978; Taylor-Papadimitriou, 1980), activation of the immune system (De Maeyer-Guignard and De Maeyer, 1985) and induction of mRNA synthesis of various genes (Friedman *et al.*, 1984; Einat *et al.*, 1985; Ozato *et al.*, 1985). Low levels of IFN are detectable in several organs such as the spleen, kidney, liver, peripheral blood leukocytes (Tovey *et al.*, 1987) and placenta (Duc-Goiran *et al.*, 1985) of normal individuals, suggesting that IFN functions in normal physiology (Bocci, 1985). IFN has also been found in the blood of patients with autoimmune disease (Hooks *et al.*, 1979), and sometimes IFN may even cause diseases in animals (Gresser, 1982; Vilcek, 1984). IFN, therefore, seems to play a more important role in normal physiology and in diseases than was previously suspected. Since IFN interacts with various systems in the body and functions in an integrated manner, investigations using intact animals would seem to be important in elucidating the roles of IFN.

Transgenic mice are very useful in analyzing the functions of a gene either at the tissue level or at the body level (Gordon, 1983; Palmiter and Brinster, 1985). Expression of a gene can be controlled by ligating the gene downstream of an appropriate enhancer-promoter. In fact, in many cases, the expression of a transgene has been shown to be strictly tissue- and developmental stage-specific (Palmiter *et al.*, 1982; Magram *et al.*, 1985; Bucchini *et al.*, 1986). Moreover, one can choose the mode of expression of the gene, i.e. either constitutive or inducible, by selecting the enhancer-promoter. These characteristics of the transgenic mouse make it possible to examine the effect of a gene on a specific tissue or a whole body, and even on an embryo at a specific developmental stage, if necessary. In this report, transgenic mice carrying an extra mouse IFN-β gene under the control of an inducible enhancer-promoter have been constructed, and the effects of the expression of this gene on the animals were examined.

Results

Production of transgenic mice expressing mouse IFN-β

A plasmid for IFN expression was constructed by inserting mouse IFN-β cDNA downstream of the mouse metallothionein I (MT) enhancer-promoter region, starting from the plasmid pMK (Palmiter *et al.*, 1982). A poly(A) addition signal for the mRNA was provided by the herpes simplex virus (HSV) thymidine kinase (TK) gene ligated downstream of the IFN gene (Figure 1c). An *Eco*RI fragment from it containing the MT-IFN-TK gene was purified and injected into the male pronuclei of fertilized mouse eggs. From a total of 2210 eggs injected, 207 pups were born. Through Southern blot hybridization of DNA using IFN-β cDNA as a probe, 10 of these pups were found to carry the extra IFN gene. The copy number of the exogenous IFN gene varied from mouse to mouse; transgenic mice such as T344, T394, T467 and T581 contained only one or two copies of the exogenously introduced IFN gene, while T312, T324 and T341 contained >10 copies, as estimated by Southern blot hybridization using the endogenous IFN band as a reference (data not shown). T328, T395 and T404 transgenic mice contained intermediate numbers of the gene (4-8 copies). No direct relationship was observed between the gene dosage and the expression level (see below).

After induction of mRNA synthesis by $CdSO_4$, expression of the exogenous IFN gene was detected in the liver, the testis and less abundantly in the brain, by RNase protection assay (Figure 1a, the 551-base band). In the testis, several RNA bands slightly longer than expected from the MT mRNA cap site were detected in addition to the 551-base band. Since these mRNAs were present only in transgenic mice, it seemed likely that they were derived from the trans-

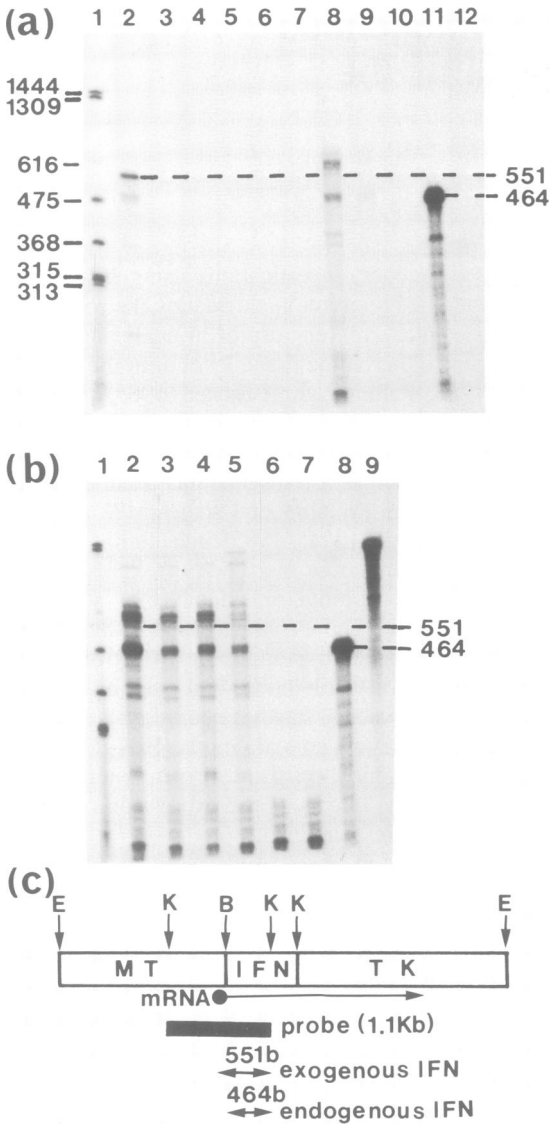

Fig. 1. Detection of mRNA specific to IFN. The mice received subcutaneous injections of $CdSO_4$ (2 mg/kg body weight) 24 and 6 h before removing the organs. (**a**) Total RNA [20 μg except 8 (10 μg) and 11 (2 μg)] from various organs of a transgenic mouse (T626, a $T395F_1$) was analyzed through RNase protection assay. The 551-base band represents RNA specific to the exogenous IFN-β gene and the 464-base band to the endogenous IFN-β gene. **Lane 1**, *TaqI* digest of pBR322; **lane 2**, liver; **lane 3**, spleen; **lane 4**, kidney; **lane 5**, lung; **lane 6**, heart; **lane 7**, seminal vesicle; **lane 8**, testis; **lane 9**, brain; **lane 10**, intestine; **lane 11**, L cells induced for IFN synthesis by infection with Newcastle disease virus; **lane 12**, tRNA. (**b**) RNase protection assay of the total RNA [20 μg except 4 (10 μg) and 8 (2 μg)] from the testes of different transgenic lines. **Lane 1**, *TaqI* digest of pBR322; **lane 2**, T609 (T344 F_1); **lane 3**, T627 (T395 F_1); **lane 4**, T730 (T328 F_1); **lane 5**, T924 (T341 F_1); **lane 6**, T581; **lane 7**, control mouse; **lane 8**, L cells infected with Newcastle disease virus; **lane 9**, RNA probe only. (**c**) Structure of the DNA injected and the RNA protected from RNase treatment. MT and TK represent the mouse metallothionein I enhancer−promoter and thymidine kinase gene, respectively, derived from the plasmid pMK (see Materials and methods). B, *BglII*/*BamHI*; E, *EcoRI*; K, *KpnI*.

gene and contained at least part of the IFN sequence. It is not known, however, whether or not these long mRNA species are functional. Peculiarly, another band corresponding to the endogenous IFN-β gene (464 base) was also detected in those transgenic mice expressing the exogenous

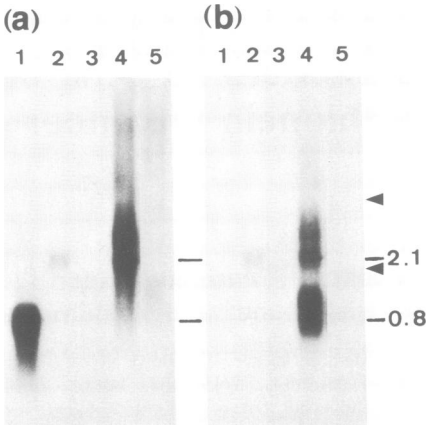

Fig. 2. Northern blot hybridization. (**a**) Poly(A)$^+$ RNA from the liver and the testis of a transgenic mouse (T1373, a $T395F_1$) which was given subcutaneous injections of $CdSO_4$ (2 mg/kg body weight) 24 and 6 h before being analyzed using IFN-β as a probe. **Lane 1**, total RNA (5 μg) from L cells induced for IFN synthesis by infection with Newcastle disease virus; **lane 2**, poly(A)$^+$ RNA (3 μg) from the liver of the transgenic mouse; **lane 3**, poly(A)$^+$ RNA (3 μg) from the liver of a normal non-transgenic mouse; **lane 4**, poly(A)$^+$ RNA (3 μg) from the testis of the transgenic mouse; **lane 5**, poly(A)$^+$ RNA (3 μg) from the testis of a normal mouse. (**b**) The same filter as in (a) was rehybridized with the TK probe (a 1.7-kb *BglII*−*PvuII* fragment from the plasmid pMK). The migration positions of 28S and 18S rRNA are shown by arrowheads; those of the transgene mRNA (2.1 kb MT-IFN-TK fusion mRNA) and the endogenous IFN-β mRNA (0.8 kb) are also shown.

IFN gene (Figure 1a and b). We therefore further analyzed RNA synthesis by Northern blot hybridization to see whether or not the 454-base band represents the endogenous IFN-β mRNA.

As shown in Figure 2a, only one band (2.1 kb), as expected for mRNA specific to the transgene, was detected in the liver with IFN-β cDNA as the probe, while no band corresponding to the endogenous IFN gene could be detected. Thus, the 464-base band observed in the RNase protection assay was demonstrated not to be the product of the endogenous IFN-β gene. The band could be an artefact of the RNase protection assay or a truncated mRNA with a starting site ~90 bases downstream of the authentic cap site of the MT-IFN fusion gene. In the testis, an additional band with a higher mol. wt (3.0 kb) was observed, probably representing a read-through product.

When the same Northern blot was probed with the TK gene, the 2.1-kb band which was detected with the IFN probe was again detected (Figure 2b). Thus, the 2.1-kb mRNA was proven to be derived from the MT-IFN-TK fusion gene with the correct initiation and termination sites. In the case of testicular cells, some additional bands of both higher and lower mol. wts could be seen, suggesting the presence of some aberrant molecules.

Antiviral activity was detected in the sera of six mouse lines, at levels ranging from 15 to 430 IU/ml (Table I); the activity was neutralized by the international reference antibody to mouse IFN-(α/β). In contrast, sera from 13 non-transgenic siblings showed <7 IU/ml, with one exception showing 14 IU/ml. Thus, it is suggested that the antiviral activity in the serum was due to the expression of the introduced IFN gene in these transgenic mice. The level of IFN activity in the serum varied markedly among indi-

Table I. Characteristics of transgenic mice

Progenitor mouse	Trans-mission[a]	Fertility of the F_1 mouse	IFN titer[b] (IU/ml)	
			−anti-IFN	+anti-IFN
T312 (♂)	0/38		64	−[c]
T324 (♂)	0/21		53	−
T328 (♀)	15/31	F_1 (♂) sterile [d]	430	<6
		F_1 (♀) fertile	<6	−
T341 (♀)	4/19	F_1 (♂) sterile	<6	−
		F_1 (♀) fertile	−	−
T344 (♀)	11/22	F_1 (♂) sterile	15	−
		F_1 (♀) fertile	25	<9
T394 (♀)	0		<25	−
T395 (♀)	11/17	F_1 (♂) sterile	130	<5
		F_1 (♂) sterile	210	<8
		F_1 (♀) fertile	−	−
T404 (♂)	0[e]		>320	−
T467 (♂)	0/6		6	−
T581 (♂)	0/33		8	−
Control			<7[f]	−

[a]Number of offspring with exogenous IFN gene/total number of offspring.
[b]IFN titer in serum was measured using blood samples from mice given subcutaneous injections of $CdSO_4$ (1 or 2 mg/kg body weight) 24 and 6 h before blood removal
[c]−, not tested.
[d]Male sterility was tested by keeping each mouse with three fertile ICR females in a cage for at least 1 month, starting at the age of 6 weeks.
[e]This mouse died during pregnancy.
[f]IFN titer in the serum of 13 non-transgenic siblings was measured, 11 showing <7 IU/ml (three females and eight males), while one (male) had 7 IU/ml, and one (male) 14 IU/ml.

viduals, even within the same transgenic line (Table I). The reason for this variation is unknown with neither sex (Table I) nor age (data not shown) showing any significant correlation.

Male sterility and expression of the IFN gene in the testis

During the course of these experiments, it was noted that all males of F_1 generation (seven so far) were sterile (Table I). In addition, one of the male progenitor mice (T404) was also sterile. Though the remaining four progenitor males were fertile, they were considered to be mosaics with respect to the exogenous IFN gene, since they did not transmit the transgene to their offspring. In fact, the copy number of the exogenous IFN gene was found to be much smaller in the testis of one of these male progenitors (T581) than in the tail (data not shown). On the other hand, female mice were fertile and transmitted the transgene to their offspring normally.

Male sterility corresponded well with whether or not expression of the exogenously introduced IFN gene took place in the testis; in the sterile mice, the IFN gene was expressed ($T328F_1$, $T341F_1$, $T344F_1$, $T395F_1$), whereas in the fertile mouse (T581), it was not (Figure 1b).

Expression of the exogenous IFN gene was oberved in the testis as early as 3 weeks after birth, when the testis is still immature, the level of expression remaining unchanged up to 15 weeks (Figure 3). It should be noted that expression in the testis was constitutive, a high level of mRNA being observed without induction (Figure 3, lanes 3 and 4). Enhancement by Cd^{2+} was not observed. In contrast, expression in the liver was observed only when mice received injections of $CdSO_4$ (lanes 7−10). Induction by the heavy metal was >10-fold in this organ.

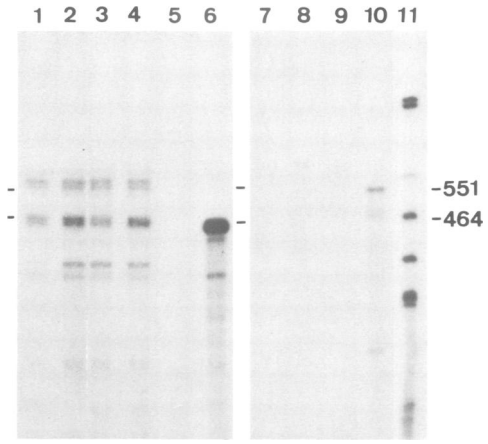

Fig. 3. Detection of mRNA specific to IFN in the testis and the liver. Total RNA (20 μg) from the testes (**lanes 1−5**) and livers (**lanes 7−10**) of transgenic mice (T395 F_1 or F_2) were analyzed by RNase protection assay. **Lanes 1** and **7**, 3-week-old transgenic mouse; **lanes 2** and **8**, 6-week-old transgenic mouse; **lanes 3** and **9**, 15-week-old transgenic mouse; **lanes 4** and **10**, 15-week-old transgenic mouse into which $CdSO_4$ (2 mg/kg) was injected; **lane 5**, 15-week-old normal mouse into which $CdSO_4$ (2 mg/kg) was injected; **lane 6**, L cells induced for IFN synthesis by infection with Newcastle disease virus; **lane 11**, *TaqI* digest of pBR322.

Table II. IFN activity and TK activity in the testis

Transgenic line	Cd^{2+}	IFN titer (IU/ml)		TK activity (pmol/h/μg prot.)
		−anti-IFN	+anti-IFN	
T328	+	80	<10	NT
	−	64	<10	3.04
T341	+	NT	NT	NT
	−	5.2	NT	0.42
T344	+	220	<10	NT
	−	130	<10	1.83
T395	+	130	<10	NT
	−	83	<10	2.75
Control	+	<5	NT	NT
	−	<5	NT	0.32 ± 0.07 (n = 4)

One side of the testis was removed either without any treatment of the mouse or after subcutaneous injections of $CdSO_4$ (2 mg/kg) 6 and 24 h before removal, and a homogenate was prepared with 1 ml of Eagle's minimum essential medium using a Dounce homogenizer. IFN titer of the supernatant fraction was measured. TK activity was measured as described in Materials and methods.
NT, not tested.

Synthesis of IFN protein was demonstrated by measuring the IFN activity in homogenates of the testis (Table II). Substantial levels of IFN activity were observed in the testes of these transgenic mice, whereas none was detected in the control non-transgenic siblings. The level of production was independent of heavy metal induction, in agreement with the findings on RNA synthesis. In these mice, the TK gene was

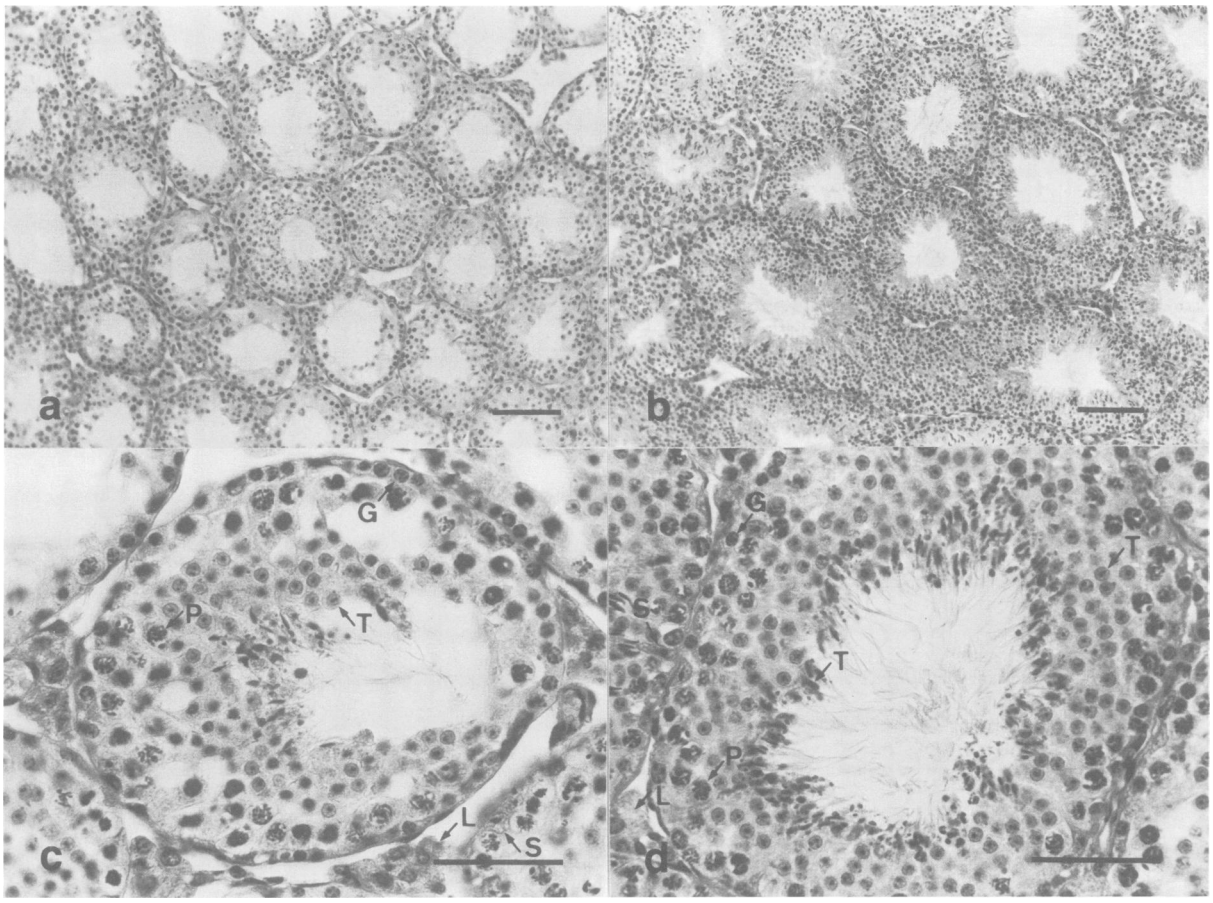

Fig. 4. Photomicrographs of testicular cross-sections. Mice were killed at the age of 6 months, and the testes fixed in Bouin's fluid. The paraffin section (5 μm) stained with hematoxylin and eosin was examined under a light microscope. **(a,c)** Seminiferous tubules from a sterile transgenic mouse (T626, an offspring of T395). Degeneration of seminiferous epithelium and shrinkage of seminiferous tubules were noted. **(b,d)** Age-matched normal control mouse. G, spermatogonium; P, spermatocyte at the pachytene stage; T, spermatid; L, Leydig cells; S, Sertoli cell. The bar indicates 100 μm (a,b) or 50 μm (c,d).

also shown to be expressed, as expected from the mRNA structure. TK activity in the testis was 5−10 times higher in transgenic mice, such as those of the T328, T344 and T395 lines, than in normal non-transgenic mice (Table II). In one of the sterile transgenic mice (T341), however, the expression level was not significantly higher than the endogenous level of the non-transgenic control. IFN activity, however, though low (52 IU/g tissue), was clearly detected in the testis. This suggested that IFN exerted deleterious effects on spermatogenesis.

Histology of the male genital organs of transgenic mice

The testes of F_1 males were found to be severely involuted. The weight of the testis was about half that of normal control mice (80−130 mg versus 200−300 mg). Sperms in the epididymis of a mouse (T626, an offspring of T395) were very much reduced in number when compared to the normal mouse (3.0×10^4 versus 3.3×10^7). On the other hand, the weights of the prostate and the seminal vesicle were normal, suggesting normal androgen levels.

Microscopy of sections of the testes of sterile transgenic mice revealed gross disorganization and degeneration of germ cells in seminiferous tubules. Representative histologic sections are shown in Figure 4. The seminiferous tubules looked atrophied, and the number of spermatocytes and maturing spermatids was reduced. A fairly large number of degenerating spermatids could be observed (Figure 4c). Abnormal spermatocytes at the pachytene stage were also observed, but less frequently. In contrast, spermatogonia and spermatocytes in the early stages of meiotic prophase were apparently normal, though somewhat reduced in number. Sertoli cells also seemed to be normal. No inflammatory cells could be observed. Abnormal changes were not marked in the testes of 3-week-old mice, in which only small numbers of spermatids and maturing sperms were present. By 6 weeks, with sexual maturity, the abnormalities gradually became evident (unpublished observation).

Discussion

In many of our transgenic mice, the extraneous mouse IFN-β gene ligated downstream of the MT enhancer−promoter was expressed in the testis, and male mice became sterile due to inhibition of spermatogenesis. Since expression of the exogenous IFN gene in the testis paralleled sterility, it seems most likely that IFN is directly involved in the inhibitory effect, though the possibility that factors induced by the IFN exerted the effect cannot be ruled out. The possibility that an insertional mutation causes male sterility seems highly unlikely, since several independent transgenic lines showed the same phenotype.

Another point worth mentioning is a possible effect of the HSV TK gene on spermatogenesis. Though the TK gene is not harmful to cultured cells, it is theoretically possible that this gene could exert adverse effects on testicular cells. In our DNA construct, the IFN cDNA was inserted upstream of the translation initiation site of the TK gene, the TK structural gene remaining intact. In fact, a 2.1-kb MT-IFN-TK fusion mRNA and very high TK activity in the testes of some transgenic mice were detected. Thus, the TK gene was expressed. Nevertheless, in a mouse from one of the sterile transgenic lines, T341, the TK activity in the testis was not significantly higher than the endogenous TK activity of the non-transgenic brother. Even in this transgenic mouse, however, IFN activity was clearly demonstrated in the testis. These observations strongly suggest that TK activity had nothing to do with the sterility. In relation to this, transgenic mice carrying the HSV TK gene with a mouse MT enhancer−promoter were reported by Palmiter *et al.* (1982, 1984), the males being shown to be normally fertile in spite of very high TK activity in the liver (up to 71 times as active as endogenous TK). Though there was no description of the TK activity in the testis, the TK gene should presumably be expressed in this organ, since we utilized in our experiments the same part of the enhancer−promoter of the plasmid pMK as they used. As an exception, one of the transgenic lines (MyK-103) carrying the TK gene suffered from severe transmission ratio distortion of the transgene in the males, but this was probably due to an insertional mutation induced by integration of the TK gene into a gene essential for spermatogenesis (Palmiter *et al.*, 1984). These observations, therefore, support our contention that expression of the HSV TK gene is not the cause of the observed male sterility.

Though a large number of transgenic mice carrying various kinds of genes have been reported so far, few of them demonstrated male sterility. Male transgenic mice carrying the major histocompatibility complex class II antigen gene were shown to be either infertile or did not transmit the injected gene to progeny (Pinkert *et al.*, 1985). Female mice of the transgenic lines were fertile. Since insertional mutation seemed unlikely, a possible explanation is that abnormal expression of the $E\alpha^d$ gene on sperm cells results in destruction of the sperm carrying the gene. IFN-β is known to induce the major histocompatibility complex class II antigen, though the efficiency is not as high as that of the IFN-γ (Dolei *et al.*, 1983; Rosa *et al.*, 1983). Whether or not the major histocompatibility complex class II antigen is involved in the infertility observed in our transgenic mice remains to be seen.

The presence of degenerating cells in the testis is interesting, since the cell growth-inhibitory effect of IFN is usually not cytocidal (Taylor-Papadimitriou, 1980; our unpublished observation). Degenerating spermatocytes at the pachytene stage and spermatids were observed. Therefore, cells at later stages of sperm maturation appear to be affected. These effects are clearly different from the known effects of IFN on cultured cells: G_0-G_1 arrest and prolongation of the S + G_2 phase (Taylor-Papadimitriou, 1980). No inflammatory cells were observed in the testis, thereby excluding the possibility that these tesicular cells were destroyed by immunological reactions. The absence of inflammation is clearly different from the case in which mouse IFN-γ linked to the human insulin promoter was expressed in the islets of Langerhans (Sarvetnick *et al.*, 1988). At present, we do not know the exact cell population in which IFN is expressed; therefore, we do not know whether only cells which express the IFN gene are killed or whether cells at specific stages of sperm maturation, such as spermatocytes and spermatids, are extraordinarily sensitive to a toxication of IFN.

IFN activity in the serum was detected only after heavy metal induction. Thus, IFN produced in the testis did not appreciably contribute to IFN activity in the serum; other organs, such as the liver, presumably being responsible for the activity. The level of IFN in the serum did not closely correlate with sterility; progenitor males, such as T312 and T324, which showed moderate levels of IFN in the sera after heavy metal induction, were fertile, whereas F_1 mice with very low serum IFN titers (T341 and T344 lines) were sterile. It may be presumed that these fertile progenitor males were mosaics in which the exogenous IFN gene was absent in the testis. Continuous production and accumulation of IFN in the testis, therefore, seems to be the dominant factor in the inhibition of spermatogenesis. We are currently examining the effects of continuous induction of the IFN gene by a heavy metal. Those used in the present study as well as other transgenic mouse lines carrying extra mouse IFN genes will be highly useful in analyzing the roles of IFN in diseases and normal physiology.

Materials and methods

Production of transgenic mice

A plasmid, pMK-Muβ (Kawade *et al.*, 1987), was constructed by inserting mouse IFN-β cDNA (Higashi *et al.*, 1983) into the *Bgl*II site of the plasmid pMK (Palmiter *et al.*, 1982) (kindly provided by Dr R.D.Palmiter) downstream of the mouse MT gene enhancer−promoter region and upstream of the HSV TK gene. For injection, an *Eco*RI fragment containing the MT-IFN-TK gene was separated from the pBR322 fragment by agarose gel electrophoresis. It was then dissolved in 10 mM Tris−HCl (pH 7.5)/0.5 mM EDTA at a concentration of 10 or 2.5 μg/ml, and microinjected into the male pronuclei of fertilized mouse eggs (C3H/He × C57Bl/6J F_1). Embryos were collected and cultured as previously described (Iwakura and Nozaki, 1985), and mice were kept in laminar flow racks.

Detection of transgene

The transgene was detected through dot-blot hybridization using DNA prepared from the tails of mice (Hogan *et al.*, 1986). For Southern blot analysis, DNA samples (10 μg) were digested with restriction enzymes, and after electrophoresis on 0.8% agarose gel, were transferred to nitrocellulose membranes. Hybridization was carried out at 42°C with nick-translated IFN-β cDNA (1×10^8 c.p.m./μg), after which membranes were washed three times with 2 × SSC−0.1% SDS at room temperature and then three times with 0.2 × SSC−0.1% SDS at 50°C (Thomas, 1980).

Detection of mRNA specific to IFN

Messenger RNA was detected using RNase protection assay (Melton *et al.*, 1984) or Northern blot hybridization (Thomas, 1980). For RNase protection assay, 1.1 kb anti-sense [^{32}P]RNA (3.0×10^8 c.p.m./μg) was made as a probe by SP 6 RNA polymerase using the *Kpn*I fragment (1.1 kb) from pMK-Muβ which had been subcloned into the pGEM^{TM}-4 (Promega Biotec) as a template. Total RNA was prepared (Chirgwin *et al.*, 1979) from various organs, and hybridized with the RNA probe (5×10^5 c.p.m.) at 45°C overnight, followed by digestion with RNase (40 μg/ml RNase A and 2 μg/ml RNase T_1) at 30°C for 1 h. After denaturation in formamide, the digest was electrophoresed on polyacrylamide gel (4%) in the presence of 7 M urea. For Northern blot hybridization, poly(A)$^+$ RNA (3 μg), purified using oligo(dT)−latex (Japan Synthetic Rubber Co. Ltd; Kuribayashi *et al.*, 1988), was electrophoresed on 1.3% agarose gel containing formaldehyde, transferred to a nitrocellulose membrane and hybridized with ^{32}P-labeled IFN-β or TK gene probe (1×10^9 c.p.m./μg) at 42°C. The filter was washed with 2 × SSC−0.1% SDS three times at room temperature then three times with 0.2 × SSC−0.1% SDS at 45°C.

IFN assay

The IFN activity was measured on mouse L cells by the cytopathic effect reduction method using vesicular stomatitis virus (Yamamoto and Kawade, 1980). NIH mouse reference IFN (G002-905-511) was used as a standard and the IFN titer was expressed in international reference units (IU). IFN activity was neutralized using NIH international reference antibody to mouse IFN-(α/β) as previously described (Kawade, 1980).

TK assay

The TK activity was measured by the conversion of [^{3}H]thymidine into TMP (Brinster *et al.*, 1982). A cell lysate was prepared by homogenizing the testis with a Dounce homogenizer in a buffer (10-fold volume of the tissue weight) consisting of 10 mM Tris−HCl, pH 7.4, 10 mM KCl, 2 mM $MgCl_2$, 10 mM 2ME, 50 mM ϵ-aminocaproic acid, 1 mg/ml BSA and 1 mM ATP, the 30 000 *g* supernatant fraction being used for the assay. To a 20-μl reaction mixture [150 mM Tris−HCl, pH 7.5, 10 mM ATP, 10 mM $MgCl_2$, 25 mM NaF, 10 mM 2ME, 5 μCi [^{3}H]thymidine (1.67 Ci/mmol)], 20 μl of the cell lysate containing 10 or 20 μg protein was added, and the reaction was carried out at 37°C for 1 h. [^{3}H]TMP production was measured by adsorption on to DE-81 paper and subsequent scintillation counting.

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