Excess Type I Interferon Signaling in the Mouse Seminiferous Tubules Leads to Germ Cell Loss and Sterility

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Anne-Pascale Satie†1, Severine Mazaud-Guitton†1, Isabelle Seif‡, Dominique Mahé‡, Zhiguo He‡, Guilhem Jouve‡, Bernard Jégou‡, and Nathalie Dejucq-Rainsford‡2

From the †INSERM, Unité 625, Institut Fédératif de Recherche 140, Université de Rennes 1, F-35042 Rennes, France and the ‡Centre National de la Recherche Scientifique, Université Paris-Sud, EA 3544, Faculté de Pharmacie, F-92296 Châtenay-Malabry, France

Type I (α and β) interferons (IFNs) elicit antiproliferative and antiviral activities via the surface receptor IFNAR. Serendipitous observations in transgenic mice in 1988 strongly suggested that IFNα/β overexpression in the testis disrupts spermatogenesis. Here, we compare a new mouse strain transgenic for IFNβ (Tg10) and a sister strain lacking the IFNAR1 subunit of IFNAR (Tg10-Ifnar1−/−), both strains expressing the transgene in the testis. The main source of IFNβ RNA was the spermatid population. Importantly, the Tg10 mice, but not the double mutant Tg10-Ifnar1−/−, showed altered spermatogenesis. The first IFNAR-dependent histological alteration was a higher apoptosis index in all germ cell categories apart from non-dividing spermatogonia. This occurred 3 weeks after the onset of IFNβ production at postnatal day 20 and in the absence of somatic cell defects in terms of cell number, expression of specific cell markers, and hormonal activities. Several known interferon-stimulated genes were up-regulated in Tg10 Sertoli cells and prepachytene germ cells but not in pachytene spermatocytes and spermatids. In concordance with this, pachytene spermatocytes and spermatids isolated from wild-type testes did not display measurable amounts of IFNAR1 and phosphorylated STAT1 upon IFNβ challenge in vitro, suggesting hypersensitivity of these cell types to IFN. At day 60, Tg10 males were sterile, and Sertoli cells showed increased amounts of anti-Mullerian hormone and decreased production of inhibin B, both probably attributable to the massive germ cell loss. Type I interferon signaling may lead to idiopathic infertilities by affecting the interplay between germ cells and Sertoli cells.

The testis is a complex organ encompassing two functional compartments. (i) The seminiferous tubules, in which spermatogenesis takes place, are composed of the different generations of germ cells closely associated with the nursing Sertoli cells. The spermatogonia undergo mitosis and differentiate into spermatocytes. The primary spermatocytes process through preleptotene, leptotene, zygotene, pachytene, and diplotene meiotic steps to generate secondary spermatocytes. Subsequently, the secondary spermatocytes enter the spermatid stages and undergo dramatic morphological changes, finally differentiating into mature spermatozoa. (ii) The interstitial tissue, which surrounds the seminiferous tubules, comprises mainly the testosterone-producing Leydig cells as well as a few macrophages and fibroblasts and the blood and lymphatic vessels. Testis functions are tightly regulated by paracrine, autocrine, and endocrine pathways (1).

Interferons (IFNs) are a family of cytokines with pleiotropic biological effects mediated by hundreds of responsive genes. Following their discovery as potent antiviral agents, IFNs have been shown to be crucial regulators of cell growth and differentiation, apoptosis, angiogenesis, and immune cell activation (2, 3). Three classes of IFN have been identified, designated types I–III (4). Each class of IFN binds to a distinct receptor and induces specific signaling pathways, leading to the activation of different but overlapping sets of genes. Type I IFNs consist of multiple species, the main protagonists being IFNβ and the large group of IFNα subtypes (5). Type I IFNs are produced by most differentiated cells in response to infection by a virus. The common receptor of type I IFNs, the IFN α/β receptor, is composed of two subunits, IFNAR1 and IFNAR2. Upon binding of type I IFNs to their receptor, the JAK-STAT signal transduction pathway is activated (3), leading to the expression of over 300 interferon-stimulated genes (ISGs)3 (6, 7).

In the male reproductive tract, we and others have previously shown that when exposed to a viral stimulus, the testis mounts a robust antiviral defense by expressing high concentrations of type I IFNs and IFN-induced proteins (8–12). Although this strong innate immune response is likely to be critical in impairing viral replication, it may also impact upon the normal functioning of the testis. In line with this, intraperitoneal injections of IFNα have been shown to induce impaired spermatogenesis in rats (13), whereas in infertile men, the presence of high levels of IFNα was detected in the semen (14). In 1988, two transgenic studies reported sterility in male mice overexpressing IFNα or IFNβ (15, 16). However, in the IFNβ transgenic study (16), the inclusion of the herpes simplex virus 1 (HSV1) thymidine kinase (TK) coding sequence in the IFNβ expression cassette was likely to be involved in the sterility because HSV1-TK was subsequently reported to induce male infertility (17–19). In the study by Hekman et al. (15), four independent IFNα1 transgenic mice (two males and two females) were obtained, carrying a tandem arrangement of the IFNα1 expression plasmid. The two F0 males appeared to be sterile and showed testicular atro-

† Both authors contributed equally to this work.

‡ To whom correspondence should be addressed: INSERM U625, Campus de Beaurieu, F-35042 Rennes, France. Tel.: 33-2-2323-5069; Fax: 33-2-2323-5055; E-mail: nathalie.dejucq-rainsford@inserm.fr.

§ The abbreviations used are: ISG, interferon-stimulated gene; TK, thymidine kinase; Pn, postnatal day n; PCNA, proliferating cell nuclear antigen; AR, androgen receptor; AMH, anti-Mullerian hormone.
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The IFNAR1 knock-out strain (Ifnar1−/−) created by Michel Aguett on a 129S2/SvPas background (21) was first back-crossed 10 times to the C3H/HeOuJ strain. Back-cross males were mated to Tg10 females and again to F1 females to give F2 Tg10 progeny knock-out for the Ifnar1 gene. The F2 Tg10-Ifnar1−/− double mutants of both sexes proved to be fertile between 2 and 9 months of age. The following breeding schemes were then tested. First, brother-sister mating gave Ifnar1−/− progeny homozygous for the Tg10 transgene. These mice were viable and fertile, indicating that the genomic alterations in Tg10 mice were not likely to affect the present study. A homozygous Tg10-Ifnar1−/− colony was established. Second, crosses between homozygous Tg10-Ifnar1−/− males and C3H/HeOuJ females gave sterile F1 males. All of this showed that the sterility phenotype was stable and transmitted by both sexes.

Colonies of the four strains Tg10, Ifnar1−/−, Tg10-Ifnar1−/−, and wild type (WT) were housed under standard conditions in transgenic facilities of the Universities of Rennes I and Paris-Sud (France). All strains were maintained by brother-sister mating, with maternal transmission of the Tg10 transgene. They were fed a standard rodent chow, provided with tap water, maintained on a 12 h/12 h light-dark cycle, and monitored for specific pathogens according to the recommendations of the Federal Service for European Laboratory Animal Science Associations. All procedures involving animals were conducted in conformity with institutional guidelines in compliance with national and international laws and policies (Council Directive 87-848 of 19 October 1987, French Ministry of Agriculture and Forestry).

Assessment of Fertility—Male mice (WT, Tg10, Ifnar1−/−, and Tg10-Ifnar1−/−) of varying ages (from 6 to over 12 weeks) were housed with 8–12-week female WT mice (1:1). Courtship and copulatory patterns were normal in all individuals. Females were sacrificed for caesarean section at 15–18 days postmating, and the size of the litter was recorded (embryonic days 10–18).

Tissue Collection—Testes and epididymides were weighted and either frozen in liquid nitrogen and stored at −80 °C or fixed either in Bouin fixative or in 4% paraformaldehyde-phosphate-buffered saline (PBS) (pH 7.2). For in situ hybridization studies, animals were perfused with 4% paraformaldehyde-PBS (pH 7.2), and tissues were then fixed overnight at 4 °C in the same fixative, cryoprotected in 30% sucrose-PBS, embedded in Tissue-Tek OCT compound (Miles Laboratories, Elkhart, IN), cut into 8-μm-thick sections, and stored at −20 °C.

Sperm Analysis—For sperm counts in epididymis, the organ was first minced with scissors in 1 ml of 0.15 M NaCl containing 0.05% Triton X-100. After homogenization using six rounds of sonication (12 kHz), the cell suspension was loaded onto a Malassez hemocytometer. Sperm heads were counted in duplicate. For sperm motility and morphology analysis, epididymides were collected from 45-day-old WT and Tg10 mice. Caudal epididymides were minced in 50 μl of prewarmed M16 medium (Sigma-Aldrich), and sperm were allowed to swim out by incubation at 37 °C under 5% CO₂ for 15 min. A 20-μl aliquot was spotted onto a glass slide and covered with a 22 × 22-mm cov-
erslip. A total number of 100 sperm (both motile and immotile) were scored using differential interference contrast microscopy at ×400 magnification. The sample was counted by two independent experimenters, and the average was taken. For sperm morphology, a 30-μl aliquot of the cauda sperm preparation was fixed with an equal volume of 4% paraformaldehyde at 4 °C for 20 min and spread onto a slide. The epididymal sperm smears were stained with Harris-Shorr solution (VWR, Fontenay-sous-Bois, France). At least 100 sperm were counted for each genotype; for each spermatozoon, the head, midpiece, and tail morphologies were classified according to the Wyrobek and Bruce criteria (22).

**Immunohistochemistry, TUNEL, and Cell Count**—Immunohistochemical staining was performed using a standardized indirect streptavidin–biotin peroxidase method, as described previously (23). Following antigen retrieval (1 mM EDTA, pH 9.0, at 95 °C for 40 min or 10 mM citrate, pH 6.0, at 100 °C for 5 min) and blockage of nonspecific sites (1-h incubation at room temperature to remove debris, and the supernatant was stored at −80 °C. Steroids were extracted with ethyl ether, and testosterone was measured by radioimmunoassay (Immunotech, Beckman Coulter, catalog no. IM1087).

**RNA Extraction, Reverse Transcription, and Conventional and Quantitative PCR**—RNA was isolated using the RNeasy minieextraction kit (Qiagen) and treated with DNase (Promega). Complementary DNA was obtained from 4 μg of total RNA using random hexamers and Moloney murine leukemia virus reverse transcriptase (Invitrogen). Conventional PCR was performed using Taq polymerase (Qiagen) and a Peltier thermocycler (MJ Research, Bio-Rad DNA engine) using the primers listed in Table 1. A real-time quantitative PCR assay was employed on 40 ng of equivalent RNA to study the expression profile of Ifnb1 and the IFN-inducible genes Oas1g and Mx1. Real-time PCR was performed using a 7500 real-time PCR system and predesigned TaqMan gene expression assays (Applied Biosystems) for Ifnb1 (context sequence 5′-CTCCACCGTGC-GTTTCTGCTGTGCT-3′), Mx1 (context sequence 5′-CTGTCGTAAGTCCCCATATTAA-3′), and Oas1g (context sequence 5′-TGGTTTTCTTCTTGAGACATAAT-3′; the assay does not amplify Oas1a corresponding sequences). It is noteworthy that the murine genome has eight corresponding sequences). It is noteworthy that the murine genome has eight.

| Gene name | Forward and reverse primers (5′–3′) | Fragment size |
|-----------|------------------------------------|---------------|
| Ccl5 (Rantes) | GATCACTCGGAGAAGCAACTCA | 310 bp |
| Cxcl10 (IP-10) | ACTGCTAAGTCCAAAATTAAA-3 | 385 bp |
| Daxx | GTGGTGGTGCAATCGTGGGC | 310 bp |
| Egf2ak2 (Pkr) | ACTGCTAAGTCCAAAATTAAA-3 | 385 bp |
| Fas | TGACGAGGCTGGTTCTTACGACATTC | 310 bp |
| Gbp2 | AGTGGTGAGGCTCAAGTGTCT | 310 bp |
| Ifnb1 | TGACGAGGCTGGTTCTTACGACATTC | 310 bp |
| Infl | TGACGAGGCTGGTTCTTACGACATTC | 310 bp |
| Igf1s | TGACGAGGCTGGTTCTTACGACATTC | 310 bp |
| Mx1 | TGACGAGGCTGGTTCTTACGACATTC | 310 bp |
| Oas1g | TGACGAGGCTGGTTCTTACGACATTC | 310 bp |
| Rnasel | TGACGAGGCTGGTTCTTACGACATTC | 310 bp |
| Rpl19 | TGACGAGGCTGGTTCTTACGACATTC | 310 bp |
| Stat1 | TGACGAGGCTGGTTCTTACGACATTC | 310 bp |
| Stat2 | TGACGAGGCTGGTTCTTACGACATTC | 310 bp |
| Tgfb10 (Trail) | TGACGAGGCTGGTTCTTACGACATTC | 310 bp |
| Xaf1 | TGACGAGGCTGGTTCTTACGACATTC | 310 bp |

**IFNβ and Inhibin B Enzyme Immunoassays**—Each testis was crushed with a micropotter in 250 μl of sample diluent. IFNβ in
testis homogenate supernatants was assayed using a sandwich enzyme immunoassay (IFNβ mouse ELISA kit KMC4041, Invitrogen). The range of detection was 15.6–1000 pg/ml with a conversion of 2.71 × 10^7 units/mg. Inhibin B was assayed using an enzyme immunoassay using a polyclonal antibody against inhibin B, with a range of detection of 1–1000 ng/ml (human/mouse/rat inhibin B enzyme immunoassay kit, RayBiotech).

In Situ Hybridization—The DNA used for the synthesis of the Ifnb1 riboprobe was obtained by RT-PCR and subcloning into the pCRII-TOPO vector (Invitrogen). Those used for synthesis of the Oas1g, Ghp2, Irf1, and Stat2 riboprobes were subcloned into pGEM-T easy (Promega). Primers are described in Table 1, and nucleotide sequences were verified by sequencing. Digoxigenin-labeled riboprobes were generated by in vitro transcription using the appropriate RNA polymerase (SP6 or T7, Roche Applied Science). In situ hybridization on frozen sections was carried out as described previously (24). For subsequent detection of germ cells and Sertoli cells using DDX4 and vimentin immunolabeling, respectively, sections were directly rinsed in PBS and incubated overnight at 4 °C with DDX4 antibody (Abcam, catalog no. ab13840; 1:200) or vimentin antibody (Abcam, catalog no. ab8545; prediluted) and thereafter with anti-rabbit secondary antibody for 2 h (IgG Alexa 594, Invitrogen; 1:500). For double immunofluorescence with anti-PCNA (dividing cells) antibody, the DDX4-labeled sections were washed, incubated overnight at 4 °C with PCNA antibody (Dako, catalog no. M0879; 1:100) and revealed with anti-mouse secondary antibody for 2 h (IgG Alexa 488, Invitrogen; 1:500). Fluorochrome-labeled sections were mounted in Vectashield containing the DNA stain DAPI (Vector Laboratories) and analyzed with a Zeiss Axio Imager M1 fluorescence microscope connected to a digital camera (Carl Zeiss).

Testicular Cell Isolation and Flow Cytometry Analyses—The isolation of Sertoli cells was achieved through enzymatic and mechanical digestions of 18-day-old mouse testes according to Ref. 26. Cells were resuspended in DMEM/Ham’s F-12 added with 5% FCS, 10 μg/ml EGF, 5 μg/ml transferrin, for 5 days at 34 °C under 5% CO2 and then recovered with a non-enzymatic cell dissociation solution (Sigma, catalog no. C5914). The isolation of premeiotic and early meiotic germ cells before the pachytene stage (up to and including the leptotene stage) was achieved through enzymatic digestions of 10-day-old mouse testes according to Ref. 26. Cells were resuspended in minimum Eagle’s medium added with 10% FCS, 1 mM pyruvate, and 6 mM lactate and cultured overnight at 34 °C under 5% CO2 before flow cytometry analysis. The isolation of meiotic and postmeiotic germ cells was achieved through enzymatic and mechanical digestions of 3-month-old mouse testes, as described previously (27). Freshly isolated cells were left to rest for 2 h in EKRB medium (enriched Krebs-Ringer bicarbonate medium with glucose and essential and non-essential amino acids) at 34 °C under 5% CO2. Flow cytometry experiments were carried out as described previously (28). The ploidy profile of the germ cell suspension was analyzed by incorporation of the fluorescent DNA dye DRAQ5 (Biotest). The purity of the isolated germ cells was assessed by staining for DDX4 (5 μg/ml), CD9 (BD Pharmingen, catalog no. 553758; 10 μg/ml), vimentin (Epitomics, catalog no. 2707-1; 2 μg/ml), and CD45 (FITC-conjugated; Beckman Coulter, catalog no. 7321148; 10 μg/ml). The analysis of the expression of the interferon α/β receptor subunits IFNAR1 and IFNAR2 was undertaken using anti-mouse IFNAR1 (Santa Cruz Biotechnology, Inc., catalog no. sc-53591; 5 μg/ml) and anti-mouse IFNAR2 (R&D Systems, catalog no. AF1083; 10 μg/ml) antibodies. The following secondary antibodies were used: anti-mouse labeled with phycoerythrin (Jackson ImmunoResearch, catalog no. 715-116-150), anti-goat labeled with phycoerythrin (Jackson ImmunoResearch, catalog no. 705-116-147), anti-rabbit labeled with FITC (Jackson ImmunoResearch, catalog no. 711-096-152), and anti-rat labeled with Alexa 488 (Invitrogen, catalog no. A11006).

Western Blot—Whole testes from 45-day-old Tg10 or WT mice as well as isolated meiotic and postmeiotic germ cells from adult WT mice were analyzed for STAT1 and phospho-rosine-STAT1 expression. The isolated germ cells (or the L929 cell line used as a positive control) were exposed to recombiant mouse IFNβ (Tebu, catalog no. 12400-1) at 10^3 units/ml for 30 or 60 min at 34 °C in EKRB medium. Tissue and cell lysis, protein quantification, electrophoresis on polyacrylamide gel (protein GeBaGel, Gene Bio-Application), and electrotransfer were performed as described previously (29). The membrane was then blocked with 5% nonfat dried milk in TBS, 0.05% Tween overnight at 4 °C and incubated with anti-STAT1 or anti-phospho-rosine-STAT1 (Santa Cruz Biotechnology, Inc., catalog nos. sc-346 and sc-7988; 1 μg/ml) antibodies in TBS-0.05% Tween with 1% nonfat dried milk for 2 h at room temperature. Blots were then washed and incubated with peroxi-dase-conjugated secondary rabbit antibody (GE Healthcare, catalog no. NA9340V; 1:10000), and immunocomplexes were detected with enhanced chemiluminescence (ECL Plus, GE Healthcare). ImageQuant analysis of the resultant band was subsequently performed.

Data Analysis—All data are presented as mean ± S.E. For each item of quantitative data, an analysis of variance followed by the appropriate post hoc test was used to compare differences between groups, as specified in each figure legend. Significance was accepted at a confidence level of p ≤ 0.05. Statistical analyses were performed using the SigmaStat 2.0 software package.

RESULTS

Phenotypic Characteristics of Tg10 and Tg10-Ifnar1−/− Mice during Postnatal Development—Transgenic mice (Tg10) (Fig. 1) and WT mice displayed similar behavior, external anatomy, life span, and female fertility. However, Tg10 males displayed hypofertility at 6 weeks, as indicated by the reduced percentage of males producing offspring and by the decreased number of fetuses per litter when compared with WT mice (Fig. 2, A and B). All Tg10 male mice were sterile at 9 weeks and thereafter (Fig. 2, A and B). In contrast, Tg10 mice with ablation of type I IFN receptor (Tg10-Ifnar1−/−), which were obtained by crossing Tg10 mice with congenic IFNAR1-null mice, displayed a similar fertility to WT mice at all time points (Fig. 2, A and B). Although epididymal sperm motility and morphology were similar in Tg10 and WT mice (Fig. 2, C and D), a decrease of epididymal sperm reserves of the Tg10 mice occurred at 6
weeks, followed by a drastic drop by 9 weeks (Fig. 2E). This
contrasts with the situation observed in Tg10-Ifnar1−/− mice,
whereas the TK polyadenylation region replaces the
instability sequence present in the native 3′-end of the Ifnb1
gene. The Ifnb1 transgene insertion is detailed
as the Tg10 locus. e2*, Prps1l3 exon 2 truncated by the transgene insertion. Dashed lines indicate a
genomic duplication attributable to transgene insertion.

Testicular and Epididymal Morphology and Histology—In
addition to adulthood (represented by 45-day- to 1-year-old
mice) several key ages of mouse testis
postnatal development
were analyzed; thus, postnatal day 10 (P10) represents the
time of the first appearance of leptotene spermatocytes, P20
the first early spermatids, P28 the first elongated spermatids,
and P35 the first spermatozoa (26, 30). Morphological
analysis of testes demonstrated that, whereas the testicular
and epididymal morphology of Tg10-Ifnar1−/− mice
were undistinguishable from the WT mice at all time points (Fig. 3, C and F versus A and D),
the testicular morphology of P60 Tg10 mice was dramatically
altered, with seminiferous tubules showing various
degrees of disorganization (Fig. 3, E versus D). Numerous
immature germ cells were present in the
epididymal lumen of P60 Tg10 mice,
indicating exfoliation of testicular germ cells (Fig. 3H). Because
there was no difference whatsoever between WT and Tg10-Ifnar1−/−, only WT and Tg10
were further compared. In accordance with the altered
testicular morphology observed, the testis weight of P60
Tg10 was half that of WT mice (Fig. 3J).
Our quantitative assessment showed that the number of cells
per seminiferous tubule of Tg10 mice was decreased by about
30% at P45 and 70% at P60 (Fig. 3K). Comparably, the
tube mean surface area was decreased by 50% at P60 onward (Fig. 3L). TUNEL experiments revealed that the percentage of
testicular germ cells in apoptosis in P45 Tg10 mice was more than
double that of WT mice, whereas at P35 it was similar to that of
FIGURE 3. Effect of the Ifnb1 transgene and Ifnar1 invalidation on postnatal testis histology and development. Testis histology from P45 (A–C) and P60 (D–F) WT (A and D), Tg10 (B and E), and Tg10-Ifnar1<sup>−/−</sup> (C and F) males shows the progressive altered spermatogenesis occurring in Tg10 testes (G–I). Immunohistochemistry for DDX4 immature germ cell marker in caudal epididymis sections of WT (G), Tg10 (H), and Tg10-Ifnar1<sup>−/−</sup> (I) shows the abnormal presence of immature germ cells in the epididymal lumen of Tg10 mice. J, mean testis weight recorded during postnatal development and in adult males shows the remarkable testis weight reduction in Tg10 males compared with WT animals at P60 (n = 8; Kruskal-Wallis analysis of variance followed by Mann-Whitney rank sum test; *, p < 0.01 versus age-matched WT males). The number of cells per tubule (K) and tubule mean surface area (L) were recorded in WT control (black bars) and Tg10 males (open bars) (n ≥ 3; Mann-Whitney rank sum test versus controls; *, p < 0.01). Scale bar, 50 μm. Error bars, S.E.
the WT (Fig. 4, A–C). A significant decrease in the number of proliferating cell nuclear antigen (PCNA)-positive spermatogonia and primary spermatocytes per tubule (Fig. 4E) was observed at P45 in Tg10 when compared with WT animals. PCNA stains mitotically dividing spermatogonia and primary spermatocytes up to and including the early pachytene stage (31). This occurred together with a trend for both PCNA-negative spermatocyte and early spermatid numbers to decrease, which became significant at P60 (Fig. 4, F and G). In contrast, the number of PCNA-negative (non-dividing) spermatogonia was not significantly modified throughout the studied period (i.e. from P35 to P180) (Fig. 4D).

The number of Sertoli cells identified by Wilms tumor protein (WT1) staining was similar in P60 Tg10 and WT mice (Fig. 5, A–C). However, at the same age, a few Sertoli cell nuclei from Tg10 mice were misplaced toward the seminiferous tubule lumen (Fig. 5C, arrows), in line with the severe disorganization of the tubules. Whereas the concentrations of inhibin B, a marker of mature Sertoli cells, was detected in Sertoli cells from both WT and Tg10 mice at P10 (Fig. 5, F and J), and its expression gradually decreased thereafter to become undetectable at P45 in both WT and Tg10 (Fig. 5, G and K). However, in contrast to WT, AMH was re-expressed in P60 Tg10 Sertoli cells (Fig. 5, H and L). Altogether, these results suggest that Tg10 Sertoli cells reached maturity and did not display morphological or cell marker (inhibin B, AR, and AMH) modifications at the onset of the altered spermatogenesis phenotype (although their full functionality cannot be presumed).

A slight non-significant Leydig cell hyperplasia was observed from P60 to 1 year of age in Tg10 (Fig. 5, M–O), possibly attributable to the seminiferous tubule shrinkage associated with germ cell loss, as previously observed in other models (33). Androgen receptor labeling of the Leydig cells appeared normal (Fig. 5, E and I). Testosterone levels in the serum of Tg10 mice before and after stimulation with human chorionic gonadotropin were similar to those of WT mice (Fig. 5P). Testicular testosterone concentrations were also comparable between Tg10 and WT mice (17.45 ± 1.86 versus 16.36 ± 6.16 ng/ml/testis, respectively), indicating that Leydig cell steroidogenesis was
not significantly affected. Accordingly, the weight of Tg10 seminal vesicles, an androgen-dependent organ, was similar to that of WT mice (data not shown).

**IFNα Temporal Expression and Producing Cells in Transgenic Mice**—IFNα expression in the testis was analyzed by ELISA, quantitative RT-PCR, and in situ hybridization. In Tg10, IFNα protein concentrations followed a rise-and-fall pattern between P20 and P60, with a mean 8.6-fold increase at P35–P45 as compared with WT (Fig. 6A). By contrast, Tg10 mice invalidated for type I IFN receptor displayed persistent high levels of IFNα concentrations from P35–P45 onward (Fig. 6A). Of note, the fact that higher levels of testicular IFNα concentrations were observed in Tg10-Ifnar1−/− when compared with Tg10 at P35–P45 probably reflects transgene homoyzogesis in the Tg10-Ifnar1−/− mice tested versus heterozygosity in Tg10. The pattern of IFNα protein expression in Tg10 testis was similar to that observed at the RNA level by real-time RT-PCR. Thus, Ifnb1 RNA significantly increased at P20 to peak at P28–P35. Its level dramatically decreased at P60 and thereafter (Fig. 6B).

**FIGURE 5. Assessment of somatic cell numbers and functionality.** A, the number of Sertoli cells per tubule in P60 mice shows no difference between WT and Tg10 testes (n = 3; Mann-Whitney rank sum test versus controls; *, p < 0.01). WT1-immunohistological labeling of Sertoli cell nuclei in P60 WT control (B) and Tg10 testes (C) shows the expected basally located (arrowheads) and abnormally located Sertoli cell nuclei toward the seminiferous tubule lumen (arrows) in the Tg10 testis. D, inhibin B concentrations were decreased in the testes of P60 Tg10 versus WT but were not statistically different at P35 (n = 4; analysis of variance followed by Fischer’s least significant difference post hoc test; *, p < 0.05). Immunohistological comparison of androgen receptor (E and I) in WT (E) and Tg10 testes (I) shows immunoreactivity in Sertoli (thin arrows), peritubular (thick arrows), and Leydig cells (arrowheads) in both WT and Tg10 testes. Labeling of the Sertoli cell marker AMH (F–H and J–L) shows progressive decline of expression in both WT and Tg10 testes (F–H and J–K) followed by reappearance in seminiferous tubules from P60 on in Tg10 testes (K and L). Immunolabeling of Leydig cells by 3β-hydroxysteroid dehydrogenase (M and N) in WT (M) compared with Tg10 testes (N), associated with assessment of the number of associated Leydig cell number per tubules (O), reveals that the apparent increase in Leydig cell density (M–O) is not statistically significant (n = 3; Mann-Whitney rank sum test versus controls; *, p < 0.01). P, in agreement, the plasma testosterone levels with or without human chorionic gonadotropin (hCG) stimulation are not statistically different in P60 Tg10 mice (open bars) and WT (black bars) (n = 3, analysis of variance followed by Fischer’s least significant difference post hoc test; *, p < 0.05). Scale bars, 50 μm (B–K) and 100 μm (L and M). Error bars, S.E.
In situ hybridization for the Ifnb1 sense RNA and co-staining of the germ cell marker DDX4 (DEAD box polypeptide 4) revealed localized transcription of Ifnb1 within the seminiferous tubules of P45 Tg10 mice (Fig. 6, E and F), whereas no Ifnb1 transcript was detected in the testes of WT mice at this age (Fig. 6, C and D). The DDX4/PCNA staining, together with the crown aspect of the Ifnb1 RNA localization around the tubules lumen, indicated spermatids (a cell type that appears in the mouse testis around P20) as being the main IFNβ-expressing cell type in Tg10 testes (Fig. 6, G–J). No up-regulated IFNβ expression was observed in (vimentin-rich) Sertoli cells and interstitial cells in Tg10 compared with WT mice (Fig. 6, K–N).

Type I IFN Receptor Expression by Seminiferous Tubule Cells—To determine the potential primary targets for IFNβ in Tg10 seminiferous tubules, we examined type I IFN receptor distribution. Because immunohistochemistry for IFNAR1 and IFNAR2 on testis sections generated recurrent diffuse background staining that blunted reliable interpretation in situ (data not shown), the expression of the type I IFN receptor subunits, IFNAR1 and IFNAR2, was investigated using flow cytometry on isolated Sertoli cells, premeiotic/early meiotic germ cells (i.e. spermatogonia and primary spermatocytes before the pachytene stage), and meiotic/postmeiotic germ cells (i.e. pachytene spermatocytes and spermatids) from WT mice. As expected, vimentin-positive Sertoli cells displayed surface expression of both IFNAR1 and IFNAR2 subunits (Fig. 7A). The premeiotic/early meiotic cell preparation was free of contaminating CD45-positive leukocytes (data not shown) and stained positive for CD9 as described previously (34) (Fig. 7B). It encompassed both diploid (2N, 2C) spermatogonia (about 70% of the population) and tetraploid (2N, 4C) mitotic spermatogonia and prepachytene spermatocytes (about 30%), as determined by ploidy analysis (data not shown). This cell population displayed a strong cell surface staining for both the IFNAR1 and IFNAR2 subunits of the type I IFN receptor (Fig. 7B). The meiotic and postmeiotic cell preparation was free of CD45⁺ leukocytes and vimentin⁺ somatic cells (data not shown). As expected, tetraploid (2N, 4C) meiotic germ cells (composed mostly of pachytene spermatocytes) and to a lesser extent haploid (1N, 1C) postmeiotic spermatids stained positive for DDX4 (Fig. 7C). In contrast to germ cells before the pachytene stage, although IFNAR2 was present within pachytene spermatocytes and spermatids, no cell surface or intracellular staining for IFNAR1 was observed in either subpopulation, suggesting the absence of functional type I IFN receptor (Fig. 7C).

In situ hybridization for the Ifnb1 sense RNA and co-staining of the germ cell marker DDX4 (DEAD box polypeptide 4) revealed localized transcription of Ifnb1 within the seminiferous tubules of P45 Tg10 mice (Fig. 6, E and F), whereas no Ifnb1 transcript was detected in the testes of WT mice at this age (Fig. 6, C and D). The DDX4/PCNA staining, together with the crown aspect of the Ifnb1 RNA localization around the tubules lumen, indicated spermatids (a cell type that appears in the mouse testis around P20) as being the main IFNβ-expressing cell type in Tg10 testes (Fig. 6, G–J). No up-regulated IFNβ expression was observed in (vimentin-rich) Sertoli cells and interstitial cells in Tg10 compared with WT mice (Fig. 6, K–N).

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STAT1 Phosphorylation Status in Tg10 Testes and in Isolated WT Meiotic/Postmeiotic Germ Cells Treated with IFNβ—The phosphorylation of STAT1 constitutes part of the canonical type I IFN signaling pathway (i.e. a complex of phosphorylated STAT1, STAT2, and un phosphorylated IRF9). Phosphoryrosine-STAT1 was detected in Tg10 but not WT testes in Western blot, and total STAT1 protein was overexpressed in the testes of Tg10 compared with those of WT mice (Fig. 8A), thus pointing to an activation of type I IFN signaling in Tg10 testes. To further investigate the apparent lack of functional IFN receptor expression by pachytene spermatocytes and spermatids (see above), these cells isolated from WT mice were exposed to recombinant IFNβ, and the phosphorylation of STAT1 was analyzed. In contrast to the L929-positive control for which STAT1 phosphorylation was detected following IFNβ exposure, no phosphorosynex-STAT1 was detected in the pachytene spermatocyte and spermatid population (Fig. 8B), further demonstrating the hyporesponsiveness of these cells to type I IFN.

Type II IFN Receptor Expression in Pachytene Spermatocytes and Spermatids—Because IFNγ is known to be involved in male germ cell apoptosis (5, 35), it was determined whether IFNγ expression was induced by G418 selection in Tg10 (Fig. 8C). The lack of IFNγ expression was confirmed by conventional RT-PCR (data not shown). Western blot analysis of the L929-positive control confirmed the phosphoryrosine-STAT1 expression in G418-stimulated cells (Fig. 8D). The expression of IFNAR2, which is responsible for the binding of IFNγ and other IFN family members, was observed at the base of the seminiferous tubules of Tg10 testes (Fig. 8D, E), whereas no IFNAR1 was detected in the testes from Tg10 mice (data not shown), testes from Tg10 mice displayed temporal expression of two ISGs, Oas1a/g and Mx1, was first compared in Tg10 and WT testes using real-time RT-PCR. Increased Oas1g expression occurred from P28 onward, thus showing correspondence in its timing of appearance with that of IFNβ in Tg10 testes. Oas1g expression then plateaued at P35 and remained high thereafter (Fig. 9A). Similarly, expression of Mx1 increased from P35 onward although to a lower level than Oas1g (Fig. 9B).

We then screened by conventional RT-PCR for the change in expression of other known ISGs in the testes of P45 WT and Tg10 mice (i.e. at the peak of IFN production and at the onset of the Tg10 germ cell loss). Whereas for Rnasel, Ccl5, Daxx, Stat1, and Fas, no obvious genotype-associated difference was observed (data not shown), testes from Tg10 mice displayed up-regulation of Gbp2, Ifj1, Stat2, Isg15, Cxcl10, Tnfα, and Xaf1 in addition to Oas1a/g and Mx1 (Fig. 9C).

In situ hybridization analysis of Ifr1, Gbp2, Oas1a/g, and Stat2 mRNAs was combined with immunofluorescence against known somatic (vimentin) and germ cell (DDX4) markers to identify IFNβ-responsive cell types in Tg10 testes (Fig. 10). Expression of Ifr1, Gbp2, and Oas1a/g transcripts was only detectable at the base of the seminiferous tubules of Tg10 testes (Fig. 10, B, D, and F) compared with WT animals (Fig. 10, A, C, and E). Immunohistochemistry with antibodies against (human) OAS1 further demonstrated labeling at the base of seminiferous tubules in P45 Tg10 mice (Fig. 10, G and H) as well as a strong staining of seminiferous tubules, including those

FIGURE 6. IFNβ temporal expression and producing cells in transgenic mice. A, IFNβ in WT (black bars), Tg10 (open bars), and Tg10-Ifnar1⁻/⁻ testes (striped bars) show increased concentrations in Tg10 and Tg10-Ifnar1⁻/⁻ testes from P20 onward (n = 6, Kruskal-Wallis analysis of variance followed by Student Newman-Keuls multiple comparison test; *, p < 0.05). B, relative ifnb1 RNA quantities in Tg10 compared with WT animals of the same age were assessed by real-time RT-PCR, without distinguishing between the sense and antisense transcripts (because the ifnb1 gene is intronless). Transcript levels started to increase in Tg10 at P20 and declined from P60 onward (n = 4, Student’s t test modified for small samples of <30; *, p < 0.05 versus transcript level in Tg10 of previous age). C–F, combined in situ hybridization analysis of ifnb1 sense RNA expression (D and F) and immunohistochemistry of the germ cell marker DDX4 (C and E) on the same sections in WT (C and D) and Tg10 (E and F). P45 testes show the up-regulation of ifnb1 RNA expression in seminiferous tubules of Tg10 testes. Comparison of double labeling for DDX4 (germ cells; red) and PCNA (germ cells up to early pachytene stage with the exception of preleptotene stage; green, orange arrows) with ifnb1 RNA localization in WT (G and H) and Tg10 testes (I and J) at P45 allows identification of DDX4-positive spermatids (open arrows) as the main ifnb1 RNA-expressing cell type in Tg10 transgenic mice. No up-regulated ifnb1 expression was observed in vimentin-positive (VIM) Sertoli cells (red, green arrows) and in the interstitium (L, Leydig cells) of Tg10 (M and N) as compared with WT (K and L). Scale bar, 100 μm (C–F) and 50 μm (G–N). Error bars, S.E.
with massive germ cell loss (stars) in P60 Tg10 (Fig. 10, I and J), indicative of Sertoli cytoplasm labeling. The Gbp2 mRNA labeling in Tg10 testes was compared with DDX4 labeling of germ cells (Fig. 10, K and L) and with vimentin labeling of Sertoli cell cytoplasm (Fig. 10, M and N). Both comparisons suggested that Gbp2-expressing cells were of Sertoli and premeiotic/early meiotic germ cell (before pachytene stage) nature. In agreement with this, it was observed that, whereas Stat2 mRNA was expressed in WT testes by a population of tubular cells located far from the basement membrane (Fig. 10, O and P), it was expressed in a larger cell population reaching those close to the basement membrane of the tubule in Tg10 testes (Fig. 10, Q and R). Altogether, these data suggest that both Sertoli and premeiotic/early meiotic germ cells are responsive to IFNβ within the testes of Tg10 mice. In contrast, ISG up-regulation was detected neither within pachytene spermatocytes and spermatids nor within the interstitial tissue.

**DISCUSSION**

This study stresses that type I interferon can affect spermatogenesis. We showed that the activation of type I IFN receptors in transgenic testes overexpressing IFNβ (Tg10) leads to an increase in the apoptosis index of various germ cell types, a depletion of germ cells, and sterility. Testicular integrity and normal fertility were maintained in Tg10 mice invalidated for the type I IFN receptor (Tg10-Ifnar1−/−). The temporal and quantitative measurement of IFNβ expression during postnatal testis development, together with the analysis of fertility and testis morphology that we undertook in Tg10 and WT mice, revealed that hypofertility and reduced epididymal sperm
count occurred at 6 weeks postnatal (i.e. about 3 weeks after the onset of gradually increasing IFN expression at P20), leading to sterility of all mice by P60.

Our analysis of the genital tract of sexually mature Tg10 male evidenced several abnormalities common to those previously reported for type I IFN transgenic mouse strains (15, 16), namely (i) a testis weight about half that of the control; (ii) atrophy of the seminiferous tubules due to disruption of spermatogenesis; (iii) reduced sperm reserves; and (iv) an absence of infiltrating inflammatory cells, suggesting that although IFN-α/H9252 is known to induce MHC class I antigens (35), acquired immune response is unlikely to be responsible for the disrupted spermatogenesis. However, there were differences between Tg10 and previous IFN transgenic mouse models. First, Iwakura et al. (16) reported a preferential loss and degeneration of pachytene spermatocytes and spermatids, whereas spermatogonia and spermatocytes in the early stages of meiotic prophase were apparently normal and only “somewhat reduced in number.” In P45 Tg10 mice, the germ cell loss was more pronounced in the population of mitotic spermatogonia and primary spermatocytes up to the early pachytene stage than in later spermatocytes and in spermatids (Fig. 4), and the loss of early germ cells remained obvious at P60 (Fig. 4) and until the age of 1 year. This difference from the study by Iwakura et al. (16) may be explained by the presence of the herpes (HSV1) thymidine kinase in the testes of their IFN-α/H9252 transgenic mice (17–19). Second, whereas the spermatogenesis alterations and occurrence of infertility were homogenous between Tg10 mice at any given age and invariably appeared at the time of maximum IFNβ testicular concentrations, Hekman et al. (15) reported highly variable time of appearance of sterility as well as heterogeneous levels of IFNoα1 expression and seminiferous tubule degeneration between individuals, including tubule calcification (which was never observed in Tg10) in the testes from the IFNoα1 transgenic strain. This may suggest that in the latter strain, the transgene expression pattern varied among males or that additional factors were at play. Altogether, these results indicate that the Tg10 strain constitutes a novel and reliable model to study the effect of type 1 IFN overexpression in the testis.

Our study is the first to address the question of the origin of the testicular dysfunction following increased type I IFN signal-
ing and the cellular mechanism at play. This was done by investigating (i) the spatio-temporal expression profiles of IFNβ in the testis; (ii) the precise testicular phenotype observed in Tg10 (e.g. cell type numbers, specific cell marker expression, including markers of somatic cell functions, and apoptosis detection); and (iii) the ability of the different testicular cell types to respond to IFN in vivo and in vitro.

We show that Ifnb1 transcripts were exclusively detected in Tg10, whereas no basal expression was evidenced in control mice using in situ hybridization. The transcripts were localized at the seminiferous tubule apex, within the spermatid populations. This cellular localization was confirmed by the profile of expression of IFNβ protein and RNA, the latter mirroring the life span of spermatids in Tg10, with increasing IFNβ concentrations from P20 when the early spermatids first appeared, peaking at P35 when the first spermatozoa occurred, and drastically decreasing thereafter, thus coinciding with the collapse of the spermatid populations. Therefore, we demonstrate that early spermatids represent the main source of IFNβ within the Tg10 testis. Importantly, because these cells do not express functional type I IFN receptor (see below), the type I IFN-induced sterility does not appear to be triggered by a synergetic interaction between IFNβ signaling and transgenic RNAs that could be assimilated to foreign duplex RNAs, known to activate some IFN-induced proteins (36).

Because our stereological analysis of Tg10 testes evidenced an increased apoptotic index for all germ cell types from P45 onward (with the exception of non-dividing spermatogonia), we investigated whether IFNβ could directly signal in germ cells and induce their apoptosis. Our results indicate that both meiotic spermatocytes (from the pachytene stage) and post-meiotic spermatids of adult WT mice lacked functional type I IFN receptor because they did not express the IFNAR1 subunits and failed to respond to IFNβ stimulation in vitro. This is consistent with our observations that (i) no up-regulation of any of the ISGs tested was ever seen within these cell populations in vivo (Fig. 10) and that (ii) in previous studies, rat meiotic and post-meiotic germ cells exposed to type I or II IFNs in vitro did not express IFN-induced proteins or transcripts (8, 37). The absence of type II IFN receptor had also been reported previously in both rat and human testicular germ cells (38, 39). Overall, our data indicate that type I IFN signaling does not operate in germ cells from the pachytene stage of spermatocytes to postmeiotic spermatids. In contrast, we found that the population of early primary spermatocytes (before the pachytene stage) and spermatogonia expressed both subunits of the IFN receptor, which is consistent with our previous data showing responsiveness of rat premeiotic germ cells to IFNγ (11) and the fact that several ISGs appeared to be induced in situ in premeiotic/early meiotic germ cells of 45-day-old Tg10 (Fig. 10). Therefore, it can be deduced that premeiotic/early meiotic germ cells can contribute to the massive germ cell loss observed in Tg10 mice. This may happen through a mix of increased apoptosis and decreased proliferation of spermatogonia. This is not excluding a contribution of the testicular somatic cells because Sertoli cells, peritubular cells, resident testicular macrophages, and Leydig cells are known to be responsive to both type I and II IFN induction (8, 11, 37, 40–42).

However, although several in vitro experiments had previously reported that type I and II IFNs decrease steroid production by porcine and rodent Leydig cells (40, 43–46), our in vivo study establishes that the excess of IFNβ within the testis of Tg10 mice did not affect Leydig cell morphology and function.

In contrast to Leydig cells and other somatic cell types, our results demonstrate up-regulation of several ISGs in the Sertoli cells of Tg10 mice. Because it is well known that germ cell development fundamentally relies on the Sertoli cell (47–49), our observations strongly suggest that Sertoli cells are involved in the germ cell degeneration process in the Tg10. This can occur through two mechanisms: (i) an early action of the Sertoli cell linked to the up-regulation of ISGs induced by the increased levels of IFNβ or (ii) a later action when the degeneration of the germ cell component had impacted Sertoli cell function per se.

As a matter of fact, we show that whereas until P45, Sertoli cells displayed normal morphology and hormonal and functional activities, they presented several abnormalities from P60 onward, including (i) a resurgence of the expression of AMH (AMH being a marker of immature Sertoli cells), which can only be observed in adult Sertoli cells in the presence of impaired spermatogenesis (32), and (ii) a decrease in production of inhibin B, a marker of Sertoli cell function (50), which declines when the number of spermatocytes and spermatids also declines (51, 52). Therefore, we conclude that Sertoli cells are most probably involved in the germ cell loss because of their ability to transduce the IFN signal and because germ cell-dependent alterations of the Sertoli cell function probably amplify the germ cell loss in a loop.

As a first step to determine the nature of IFNβ-responsive cell types in Tg10, we screened for ISG up-regulation using RT-PCR. The nine, among 14 tested, ISGs displaying up-regulated expression of their RNAs in Tg10 testis fell into diverse functional categories, including apoptosis (Tnfsf and Xaf1), antiviral (Oas1a/g and Mx1), cell signaling (Stat2 and Ifr1), ubiquitination (Isg15), GTPase (Gbp2), and chemoattraction (Cxc110) (7, 53, 54). For technical reasons (e.g. low level of expression), several of these genes could not be used for in situ localization. The four ISGs employed for the identification of target cells using in situ hybridization (Ifr1, Stat2, Oas1a/g, and

**FIGURE 10.** In situ localization of the up-regulation of four ISGs within Tg10 testes. In situ hybridization for Ifr1 (A and B), Gbp2 (C and D), and Oas1g (not distinguished from Oas1a) (E and F) shows up-regulation of these ISGs at the base of seminiferous tubules (red dotted lines in WT) in P45 Tg10 testes. At the protein level (G–J), immunohistochemistry for Oas1a at P45 (G and H) in WT (G) and Tg10 testes (H) confirmed the expression at the base of Tg10 seminiferous tubules. At P60 (I and J), expression was observed in Tg10 tubules, including those with high levels of germ cell depletion (stars) (J). Double labeling for Gbp2 RNA (K and M) and the germ cell protein DDX4 (L) or the Sertoli cell protein vimentin (N) in Tg10 testes using combined in situ hybridization and immunohistochemistry shows Gbp2-positive DDX4-positive or vimentin-negative prepachytene spermatocytes (open arrows, L and N) and Gbp2-positive DDX4-negative or vimentin-positive Sertoli cells (green arrows, L and N). In situ hybridization for Stat2 (O–R) in WT (O and P) and Tg10 P45 testes (Q and R) shows that Stat2-expressing cells are more numerous and closer to the basement membrane (red dotted line) in Tg10 testes than in control ones (P and R are higher magnifications of areas boxed in O and Q, respectively). Scale bars, 100 μm (A–J and O–R) and 50 μm (K–N).
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Gbp2) and immunohistochemistry (OASI) were up-regulated in the cell populations located at the base of the seminiferous tubules (i.e. Sertoli cells and early germ cells). A potential role of these genes in the sterility of Tg10 mice is yet to be determined. As an example, IRFI is a transcription factor that can exert antiproliferative and apoptotic effects through its target genes (55, 56) and could participate in germ cell loss. The murine OAS-RNase L system is believed to require suitable RNA activators (57) to exert its cellular effects, such as apoptosis and growth control (3). Whether this system is active in the mouse testis is presently unknown. However, new RNase L-independent pathways are emerging, through which some OAS proteins can exert antiviral, including proapoptotic, activities (58–60) in the absence of RNA activators and can even act as paracrine agents (60). We are planning to further investigate the repertoire of ISGs involved by characterizing the transcriptional response of the testis to impregnation by IFNβ, using our data base on testicular cell type-associated genes (GermOnline) (61).

A more general aspect of this transgenic model is its pathophysiological relevance to viral or bacterial infections. We and others have shown that upon in vitro exposure to virus or dsRNA, all rodent testicular somatic cells (but not germ cells) produce increased levels of type I IFN (9, 10, 12). Although the nature of the testicular producing cells differs between Tg10 and WT animals, it is worth noting that, more importantly, the maximum testicular concentrations of IFNβ in Tg10 mice (i.e. a mean of 950 pg/testis) were lower than the concentrations of type I IFN expected to be produced by rodent testicular cells in response to pathogen exposure (e.g. an estimate of about 115 ng of IFNα/β per rat testis because about 3 ng were produced per 10^6 rat Sertoli cells in 1 ml of medium (9), and the rat testis encompasses an estimated 38.4 million Sertoli cells (62)). In mice, an average of 1.6 ng of IFNβ/10^6 Sertoli cells was measured following dsRNA transfection (12). Therefore, Tg10 mice could constitute a relevant model for the analysis of the impact of increased IFN concentrations in the seminiferous tubules following infection. It can be extrapolated from our results that a sustained non-inflammatory innate response to chronic infections of the testis mediated by type I IFNs may lead to sterility. Alternatively, in cases where IFN production is temporary or restricted to a few seminiferous tubules, this may not lead to sterility but instead may have beneficial effects. Thus, IFN signaling in Sertoli and premeiotic germ cells would not only directly protect them from infection but also would indirectly prevent infection of the IFN non-responsive meiotic and postmeiotic germ cells by inducing their programmed cell death.

In conclusion, we demonstrate that the activation of type I IFN receptors can severely disrupt spermatogenesis, eventually leading to sterility. This is likely to result from the combined and reciprocal action of early germ cell categories and of the Sertoli cells because both appear to be privileged targets of type I IFN. Given that somatic testicular cells have been shown to produce high type I IFN concentrations following viral exposure, this may represent a yet unidentified mechanism of pathogen-induced infertility.

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References

1. Cheng, C. Y., Wong, E. W., Yan, H. H., and Mruk, D. D. (2010) Mol. Cell. Endocrinol. 315, 49–46
2. Goodbourn, S., Didcock, L., and Randall, R. E. (2000) J. Gen. Virol. 81, 2341–2364
3. Borden, E. C., Sen, G. C., Uze, G., Silverman, R. H., Ransohoff, R. M., Foster, G. R., and Stark, G. R. (2007) Nat. Rev. Drug Discov. 6, 975–990
4. Sadler, A. J., and Williams, B. R. (2008) Nat. Rev. Immunol. 8, 559–568
5. Pestka, S., Krause, C. D., and Walter, M. R. (2004) ImmunoL Rev. 202, 8–32
6. Der, S. D., Zhou, A., Williams, B. R., and Silverman, R. H. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 15623–15628
7. de Veer, M. J., Holko, M., Frevel, M., Walker, E., Der, S., Paranjape, J. M., Silverman, R. H., and Williams, B. R. (2001) J. Leukoc. Biol. 69, 912–920
8. Dejuç, N., Chuosterman, S., and Jégou, B. (1997) J. Cell Biol. 139, 865–873
9. Dejuç, N., Dugast, I., Ruffaut, A., van der Meide, P. H., and Jégou, B. (1995) Endocrinology 136, 4925–4931
10. Dejuç, N., Lienard, M. O., Guillaume, E., Dorval, I., and Jégou, B. (1998) Endocrinology 139, 3081–3087
11. Melaine, N., Lienard, M. O., Guillaume, E., Ruffaut, A., Dejuç-Rainsford, N., and Jégou, B. (2003) J. Reprod. Immunol. 59, 53–60
12. Starace, D., Galli, R., Paone, A., De Cesaris, P., Filippini, A., Ziparo, E., and Riccioli, A. (2008) Biol. Reprod. 79, 766–777
13. Ulusoy, E., Cayan, S., Yılmaz, N., Aktaş, S., Acar, D., and Duruk, E. (2004) Arch. Androl. 50, 379–385
14. Fujisawa, M., Fujioka, H., Tatsumi, N., Inaba, Y., Okada, H., Arakawa, S., and Kamidono, S. (1998) Arch. Androl. 40, 211–214
15. Hekman, A. C., Trappman, J., Mulder, A. H., van Gaalen, J. L., and Zwarthof, E. C. (1988) J. Biol. Chem. 263, 12151–12155
16. Iwakura, Y., Asano, M., Nishimune, Y., and Kawade, Y. (1988) EMBO J. 7, 3757–3762
17. Al-Shawi, R., Burke, J., Jones, C. T., Simons, J. P., and Bishop, J. O. (1988) Mol. Cell. Biol. 8, 4821–4828
18. Braun, R. E., Lo, D., Pinkert, C. A., Widera, G., Flavell, R. A., Palmiter, R. D., and Brinster, R. L. (1990) Biol. Reprod. 43, 684–693
19. Cai, L. Y., Kato, T., Nakayama, M., Sasa, T., Murakami, S., Izumi, S., and Kato, Y. (2009) Reprod. Toxicol. 27, 14–21
20. Seif, I., De Maeyer, E., Riviere, I., and De Maeyer-Guignard, J. (1991) J. Virol. 65, 664–671
21. Müller, U., Steinhoff, U., Reis, L. F., Hemmi, S., Pavlovic, J., Zinkernagel, R. M., and Aguet, M. (1994) Science 264, 1918–1921
22. Woyrobek, A. J., and Bruce, W. R. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 4425–4429
23. Roulet, V., Satie, A. P., Ruffaut, A., Le Tortorec, A., Denis, H., Guist’hau, O., Patard, J. J., Rioux-Leclercq, N., Gicquel, J., Jégou, B., and Dejuç-Rainsford, N. (2006) Am. J. Pathol. 169, 2094–2103
24. Mazaud, S., Guigon, C. J., Lozach, A., Coudouel, N., Forest, M. G., Cofigny, H., and Magre, S. (2002) Endocrinology 143, 4775–4787
25. Toehbosch, A. M., Robertson, D. M., Klijai, I. A., de Jong, F. H., and Groote- goed, J. A. (1989) J. Endocrinol. 122, 757–762
26. Bellvé, A. R., Cavicchia, J. C., Millette, C. F., O’Brien, D. A., Bhatnagar, Y. M., and Dym, M. (1977) J. Cell Biol. 76, 439–445
27. Bellvé, A. R. (1993) Methods Enzymol. 225, 84–113
28. Willey, S., Roulet, V., Reeves, J. D., Kergadallan, M. L., Thomas, E., McKnight, A., Jégou, B., and Dejuç-Rainsford, N. (2003) AIDS 17, 183–188
29. Guillaume, E., Evrard, B., Com, E., Moertz, E., Jégou, B., and Pineau, C. (2001) Mol. Reprod. Dev. 60, 439–445
30. Kramer, J. M., and Erickson, R. P. (1981) Dev. Biol. 87, 37–45
31. Chapman, D. L., and Wolgemuth, D. J. (1994) Int. J. Dev. Biol. 38, 491–497
32. Sharpe, R. M., McKinnell, C., Kivlin, C., and Fisher, J. S. (2003) Reproduc...
33. Jegou, B., Risbridger, G. P., and de Kretser, D. M. (1983) J. Androl. 4, 88–94
34. Kanatsu-Shinohara, M., Toyokuni, S., and Shinohara, T. (2004) Biol. Reprod. 70, 70–75
35. Israel, A., Kimura, A., Fournier, A., Fellous, M., and Kourilsky, P. (1986) Nature 322, 743–746
36. Gantier, M. P., and Williams, B. R. (2007) Cytokine Growth Factor Rev. 18, 363–371
37. Aubry, F., Habasque, C., Satie, A. P., Jegou, B., and Samson, M. (2000) Eur. Cytokine Netw. 11, 690–698
38. Kanzaki, M., and Morris, P. L. (1998) Endocrinology 139, 2636–2644
39. Schweyer, S., Soruri, A., Peters, J., Wagner, A., Radzun, H. J., and Fayyazi, A. (2000) Br. J. Cancer 89, 915–921
40. Meikle, A. W., Cardoso de Sousa, J. C., Dacosta, N., Bishop, D. K., and Samlowski, W. E. (1992) J. Androl. 13, 437–443
41. Riccioli, A., Starace, D., D’Alessio, A., Starace, G., Padula, F., De Cesaris, P., Filippini, A., and Ziparo, E. (2000) J. Immunol. 165, 743–749
42. Dal Secco, V., Riccioli, A., Padula, F., Ziparo, E., and Filippini, A. (2008) Biol. Reprod. 78, 234–242
43. Orava, M. (1989) J. Interferon Res. 9, 135–141
44. Orava, M., Cantell, K., and Vihko, R. (1985) Biochem. Biophys. Res. Commun. 127, 809–815
45. Orava, M., Voutilainen, R., and Vihko, R. (1989) Mol. Endocrinol. 3, 887–894
46. Lin, T., Hu, J., Wang, D., and Stocco, D. M. (1998) Endocrinology 139, 2217–2222
47. Skinner, M. K., Norton, J. N., Mullaney, B. P., Rosselli, M., Whaley, P. D., and Anthony, C. T. (1991) Ann. N.Y. Acad. Sci. 637, 354–363
48. Jegou, B. (1993) Int. Rev. Cytol. 147, 25–96
49. Cheng, C. Y., and Mrak, D. D. (2002) Physiol. Rev. 82, 825–874
50. de Kretser, D. M., Buzzard, J. J., Okuma, Y., O’Connor, A. E., Hayashi, T., Lin, S. Y., Morrison, J. R., Loveland, K. L., and Hedger, M. P. (2004) Mol. Cell. Endocrinol. 225, 57–64
51. Pineau, C., Sharpe, R. M., Saunders, P. T., Gérard, N., and Jegou, B. (1990) Mol. Cell. Endocrinol. 72, 13–22
52. Chawla-Sarkar, M., Lindner, D. J., Liu, Y. F., Williams, B. R., Sen, G. C., Silverman, R. H., and Borden, E. C. (2003) Apoptosis 8, 237–249
53. Ghosh, A., Sarkar, S. N., Rowe, T. M., and Sen, G. C. (2001) J. Biol. Chem. 276, 25447–25455
54. Kajaste-Rudnitski, A., Mashimo, T., Frenkiel, M. P., Guénet, J. L., Lucas, M., and Després, P. (2006) J. Biol. Chem. 281, 4624–4637
55. Kristiansen, H., Scherer, C. A., McVean, M., Iadonato, S. P., Vends, S., Thavachelvam, K., Steffensen, T. B., Horan, K. A., Kuri, T., Weber, F., Paludan, S. R., and Hartmann, R. (2010) J. Virol. 84, 11898–11904
56. Chalmel, F., Rolland, A. D., Niederhauser-Wiederkehr, C., Chung, S. S., Demougin, P., Gattiker, A., Moore, J., Patard, J. J., Wolgemuth, D. J., Jegou, B., and Primig, M. (2007) Proc. Natl. Acad. Sci. U.S.A. 104, 8346–8351
57. Wang, Z. X., Wreford, N. G., and De Kretser, D. M. (1989) Int. J. Androl. 12, 58–64