Differences in Radiation Sensitivity of Recovery of Spermatogenesis Between Rat Strains

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Previous studies with Lewis/Brown-Norway (BN) F1 hybrid rats indicated that spermatogenesis was much more sensitive to ionizing radiation than in the widely studied outbred Sprague Dawley stock, suggesting that there were genetically based differences; however, the relative sensitivities of various inbred strains had not been established. As a first step to defining the genes responsible for these differences, we compared the sensitivities of seven rat strains to radiation damage of spermatogenesis. Recovery of spermatogenesis was examined 10 weeks after 5-Gy irradiation of seven strains (BN, Lewis, Long-Evans, Wistar Kyoto, spontaneously hypertensive [SHR], Fischer 344, and Sprague Dawley). The percentages of tubules containing differentiated cells and testicular sperm counts showed that BN and Lewis were most sensitive to radiation (<2% of tubules recovered, <2 x 10^5 late spermatids per testis), Long-Evans, Wistar Kyoto, Fischer, and SHR were more resistant, and Sprague Dawley was the most resistant (98% of tubules recovered, 2 x 10^7 late spermatids per testis). Although increases in intratesticular testosterone levels and interstitial fluid volume after irradiation had been suggested as factors inhibiting recovery of spermatogenesis, neither appeared to correlate with the radiation sensitivity of spermatogenesis in these strains. In all strains, the atrophic tubules without differentiated germ cells nevertheless showed the presence of type A spermatogonia, indicating that their differentiation was blocked. Thus, we conclude that the differences in radiation sensitivity of recovery of spermatogenesis between rat strains of different genetic backgrounds can be accounted for by differences in the extent of the radiation-induced block of spermatogonial differentiation.

Key Words: ionizing radiation; spermatogenesis; rat strains; spermatogonia.

Identification and quantification of risks that particular toxicants will damage the human male reproductive system are based on results from animal model systems. Rodents have been the primary model system used in reproductive toxicology because they are small, inexpensive, and genetically well characterized. However, it is important for qualitative extrapolation to human that the mechanisms of the toxicity in the test species have the same characteristics as in the human. For quantitative extrapolation, it also is necessary to consider the doses to produce equivalent effects (Meistrich, 1992). Within a test species, the strain chosen is important because there may be quantitative differences in the response with different strains. Furthermore, there might be qualitative differences in mechanisms with different strains.

For many decades, the rat had been the primary rodent model used for reproductive toxicology. However, the mouse has been used increasingly in recent years because more genetic tools are available in this species to elucidate mechanisms, and there have been numerous studies of strain differences in effects of toxicants on spermatogenesis in mice (Bianchi et al., 1985; Meistrich et al., 1984; Spearow et al., 1999). In contrast, there have been very few reports characterizing strain differences in sensitivities of various rat strains (Delic et al., 1987; Parchuri et al., 1993; Sotomayor et al., 1996), and some of these studies often included outbred rat stocks rather than inbred strains. However, the genetic knowledge and techniques in the rat are now progressing with the sequencing of the genome (Gibbs et al., 2004), the existence of sets of recombinant inbred lines (Tabakoff et al., 2009; Voigt et al., 2008), and the ability to produce gene knockouts (Jacob et al., 2010), so that studies of strain differences in rats have the potential to lead to discovery of gene function.

Previously, we reported that there were dramatic interstrain differences in the recovery of spermatogenesis in rat testes from the chemotherapy drug procarbazine (Parchuri et al., 1993). Whereas spermatogenesis in most outbred Sprague Dawley rats was nearly completely resistant to prolonged effects of multiple injections of procarbazine on the testis, about 25% of the rats were quite sensitive to that treatment. In contrast, both Lewis and LBNF1 (F1 hybrids of Lewis and Brown-Norway [BN] inbred strains) were extremely sensitive to the same doses of procarbazine. With chemical treatment, it is not known whether the differences in sensitivity were due to...
target organ sensitivities as opposed to differences in pharmacokinetics or systemic effects.

To more specifically examine differences in target organ sensitivities, we compared the data on radiation sensitivities of different strains. Radiation is highly toxic to the human testis and 4–6 Gy can produce total loss of sperm production for about 2 years (Clifton and Bremner, 1983).

The most sensitive targets for radiation damage to the testis are the proliferating differentiating spermatogonia (A1–A4, intermediate, and B spermatogonia) (Erickson, 1976). The loss of these cells results in a progressive depletion of differentiating germ cells (Dym and Clermont, 1970). The spermatogonial stem cells (undifferentiated type A) are more resistant and can survive moderate radiation doses, and, if the dose is not too high, they can eventually produce complete recovery of spermatogenesis in resistant strains (Dym and Clermont, 1970). However, at high doses or in sensitive strains, the recovery may be incomplete or permanent testicular atrophy may occur (Kangasniemi et al., 1996). For example, LBNF1 rats were much more sensitive to prolonged spermatogenic damage from irradiation (Kangasniemi et al., 1996) than were Sprague Dawley rats, based on comparison of similar doses and endpoints gathered from the literature (Delic et al., 1987; Erickson and Hall, 1983; Huckins, 1978). Whereas LBNF1 rats showed atrophic seminiferous tubules with only A spermatogonia, indicating a block in their differentiation, Sprague Dawley rats showed progressive recovery of spermatogenesis at similar doses. A block in spermatogonial differentiation after exposure to a variety of therapeutic and environmental toxicants, including hexane-dione and dibromochloropropane, has been observed in Sprague Dawley and Fischer 344, in addition to LBNF1, rats (Meistrich and Shetty, 2003).

The radiation-induced block in spermatogonial differentiation in LBNF1 rats was not due to damage to the stem cells as they differentiated into spermatozoa after transplantation into the depleted testes of nude mice (Zhang et al., 2006). It was also not due to failure of the stem cells to proliferate, as they were actively cycling in atrophic tubules of several models with spermatogonial blocks induced by irradiation, hexane-dione, or age, but was rather due to apoptosis of these cells when they began to differentiate (Allard et al., 1995; Schoenfeld et al., 2001; Shuttlesworth et al., 2000). This block was due to damage to the somatic environment as transplanted spermatogonia from normal immature rats failed to differentiate in the irradiated testis tubules (Zhang et al., 2007). The cause of the block is not known but several candidate genes whose expression changes in somatic cells of LBNF1 rats after radiation have been identified (Zhou et al., 2010, 2011).

To extend these anecdotal observations, we directly compared the sensitivities of seven different strains or stocks of rats treated with the same doses of radiation. Strains were chosen on the basis of their usefulness in toxicological or endocrine studies, previous indications of strain differences, and the existence of recombinant inbred lines to facilitate identification of loci contributing to the phenotype. We identified very marked differences in the sensitivity of the strains to radiation.

**MATERIALS AND METHODS**

**Animals and irradiation exposure.** We examined seven strains of rats to measure the recovery of spermatogenesis after irradiation. These included five inbred strains: BN (BN/SnSHsd) and Lewis (LEW/SnSHsd) obtained from Harlan Laboratories; Fischer 344 (F344/NciCr), Wistar Kyoto (WKY/NciCr), and SHR (SHR/NciCr) obtained from Charles River Laboratories; and two outbred stocks: Long-Evans (CrI:LE) from Charles River and Sprague Dawley (Hsd:Sprague Dawley SD) from Harlan. We obtained the rats at 7 weeks of age and they were allowed to acclimatize in our facility for 1 week prior to use. Rats were housed under standard lighting (12-h light, 12-h dark) and were given food and water ad libitum. All procedures were approved by the University of Texas MD Anderson Cancer Center Institutional Animal Care and Use Committee, and the housing facilities were approved by the American Association of Laboratory Animal Care.

Rats were anesthetized with a mixture of ketamine (0.72 mg/kg) and acepromazine (0.022 mg/kg) im and affixed to an acrylic board with surgical tape; then, the lower part of the body was irradiated by a 60Co gamma ray unit (Eldorado 8; Atomic Energy Canada Ltd., Ottawa, ON, Canada). The field extended distally from a line about 6 cm above the base of the scrotum. A single dose of 5 Gy was given at a dose rate of approximately 1 Gy/min (Shetty et al., 1989). Rats were euthanized 10 weeks after irradiation; serum was collected for hormone measurements, and the testis tissue was harvested for analysis as indicated below because all tubules in Sprague Dawley rats showed recovery of spermatogenesis at this dose, another group of Sprague Dawley rats were given 6.5 Gy of irradiation, and testis tissue was harvested 10 weeks later.

For each strain/stock, (n = 5–10) irradiated rats and (n = 3) age-matched unirradiated controls were analyzed.

**Intratesticular interstitial fluid and tissue processing.** Rats were killed by an overdose of the ketamine-acepromazine mixture. Each testis was surgically excised and weighed with the tunica albuginea intact. The right testis was fixed in 10% formalin for 48 h and processed for histology, and the left testis was used for hormone measurements, and the testis tissue was harvested for analysis as indicated below because all tubules in Sprague Dawley rats showed recovery of spermatogenesis at this dose, another group of Sprague Dawley rats were given 6.5 Gy of irradiation, and testis tissue was harvested 10 weeks later.

For each strain/stock, (n = 5–10) irradiated rats and (n = 3) age-matched unirradiated controls were analyzed.

**Evaluation of spermatogenesis.** For histological analysis, the fixed right testis was cut in half, testis section was taken from the middle from one of the two pieces and then embedded in glycol methacrylate plastic (JB-4; Polysciences Inc., Warrington, PA), and 4-μm sections were cut and stained with periodic acid Schiff and hematoxylin. To evaluate the recovery of spermatogenesis from irradiation, we scored a minimum of 200 seminiferous tubules from the whole testis cross section from each animal for the most advanced germ cell stage present in each tubule. Unless otherwise stated, we computed the tubule differentiation index (TDI), which is the percentage of tubules containing three or more cells that had reached type B spermatogonial stage or later (Meistrich and van Beek, 1993). To obtain a more complete
description of the stages of differentiation present in the testis, we also determined the percentages of tubules with three or more cells reaching the leptotene spermatocyte stage or later (TDI-spermatocytes) or the round spermatid stage or later (TDI-spermatids) or with 10 or more cells reaching the elongating or elongated spermatids stage (TDI-late spermatids).

Although there are multiple subtypes of A spermatogonia in the rat testis (Chiarini-Garcia et al., 2003; van Bragt et al., 2008), they cannot be reliably distinguished in Bouin’s-fixed methacrylate-embedded sections. Therefore, we counted all type A spermatogonia and Sertoli cells in atrophic seminiferous tubule cross-sections of irradiated rat testes at ×1000 magnification (n = 3–7 per group). For samples with almost complete seminiferous tubule atrophy, cells were counted using systematic random sampling (Stereo Investigator version 8.0 software; MicroBrightField, Inc., Williston, VT), by counting A spermatogonia and Sertoli cells in 300 randomly selected 100 × 80 μm fields. Results were presented as A spermatogonia per 100 Sertoli cells. In samples with few atrophic seminiferous tubules, these tubules were identified visually using light microscopy, and all cells in the tubules were counted. A minimum of 500 Sertoli cells was counted per testis.

Testicular sperm production was evaluated by counting sonication-resistant sperm heads, which represent nuclei of step 12–19 spermatids, in testicular homogenates. An aliquot of the homogenate of the left testis was sonicated and the sperm heads were counted in a hemocytometer using phase contrast optics (Meistrich and van Beek, 1993).

**Hormone assays.** Serum testosterone and intratesticular fluid testosterone concentrations were measured using a coated-tube radioimmunoaassay kit (Coat-A-Count Total Testosterone; Cat No. TKTT1; Siemens, Los Angeles, CA) similar to procedures described previously (Porter et al., 2006; Shetty et al., 2000). Rat serum follicle–stimulating hormone (FSH) was measured by radioimmunoaassay, and luteinizing hormone (LH) was measured by a sensitive two-site sandwich immunoassay. Both FSH and LH were measured by the University of Virginia, Center for Research in Reproduction, Ligand Assay and Analysis Core, using previously described methods (Gay et al., 1970).

**Statistical analysis.** Results were presented as either mean ± SEM calculated from untransformed data or, in the case of sperm head counts, testosterone, and LH, as the mean ± SEM calculated from log-transformed data obtained from individual rats. The statistical significance of differences between two groups was determined using SPSS version 19 software (Lead Technologies, Chicago, IL) using one-way ANOVA and the Student-Newman-Keuls post hoc test with p < 0.05 being considered significant.

**RESULTS**

**Recovery of Spermatogenesis After Irradiation**

Ten weeks after 5-Gy irradiation, rats were killed and testis tissue was harvested and serum removed for hormone analysis. Whereas the control testis parenchymal weights ranged from 1.04 g (Lewis and Fischer 344) to 1.74 g (Long-Evans), irradiation markedly reduced testicular weights in all strains to between 0.29 g (Lewis) and 0.59 g (Sprague Dawley) (Fig. 1A). Expressing the parenchymal weight as a fraction of the control for each strain showed small but significant differences between strains. BN, Lewis, and Long-Evans appeared most sensitive as testicular weights decreased to between 24 and 28% of control. Fischer, Wistar Kyoto, and SHR had testicular weights of about 30% of control. Sprague Dawley was most resistant, with a testicular weight of 36% of control.

Interstitial fluid weights of control rats ranged from 0.06 g in Lewis to 0.11 g in Sprague Dawley, but no significant differences between strains were observed. Interstitial fluid weights were measured after irradiation (Fig. 1C) and showed negligible increases of only 0.01 g from the control in the Wistar Kyoto and SHR strains, marginal increases of 0.04–0.06 g in the Lewis, Fischer, and Sprague Dawley strains, but large significant increases of 0.15 g in Long-Evans and 0.22 g in the BN rats (Fig. 1D).

Despite only small differences in testis weights, the histological appearances of the testes were markedly different. Some strains, such as BN and Lewis, showed complete tubular atrophy with no differentiated germ cells present in any of the seminiferous tubules (Figs. 2A and B). However, the two strains differed in that there were large cellular interstitial spaces in BN, indicative of interstitial edema corresponding to the fluid accumulation in this strain (Fig. 1C), but not in the Lewis strain (not shown). Other strains such as SHR and Sprague Dawley showed recovery of spermatogenesis in essentially all tubules (Fig. 2C). Although late spermatids were observed in some tubules, other tubules showed incomplete recovery only to the spermatocyte or round spermatid stage (Fig. 2D).

The recovery of spermatogenesis was quantified by calculation of the TDI in histological sections (Fig. 3A). BN and Lewis were the most sensitive with less than 2% of tubules having evidence of differentiated germ cells, Long-Evans, Wistar Kyoto, and Fischer had between 50 and 75% of tubules with differentiated cells, whereas SHR and Sprague Dawley were more resistant, with evidence of differentiation in nearly all tubules. Long-Evans rats showed high variation in tubule differentiation (standard deviation: 31%), whereas the inbred strains and the outbred Sprague Dawley rats had standard deviations of < 10%.

The atrophic tubules were examined to determine whether they were a result of killing of stem spermatogonia or a block in their differentiation as previously observed with LBNF1 rats (Kangasniemi et al., 1996). The atrophic tubules observed in 5-Gy irradiated BN, Lewis, Long-Evans, Wistar Kyoto, and Fischer 344, and SHR rats contained between 2.2 and 3.9 type A spermatogonia per 100 Sertoli cells (Table 1, Fig. 2C), indicating that the stem cells were not killed but their differentiation was blocked. Although residual A spermatogonia in Sprague Dawley rats exposed to 5 Gy could not be counted because less than 2% of tubules were atrophic, at 6.5 Gy, there were atrophic tubules and they did contain 2.8 type A spermatogonia per 100 Sertoli cells.

Among the recovering tubules, there was heterogeneity in the stages to which differentiation was observed. For example, of the 54% of the tubules showing differentiation in irradiated Long-Evans rats, 1% recovered only to the B spermatogonial stage, 17% reached the spermatocyte stage, 32% recovered to the round spermatid stages, and only 5% of the tubules reached the late spermatid stage. We used these data to plot the percentages of tubules reaching each stage of differentiation or beyond (Fig. 3B). These plots revealed differences between strains in the ability of differentiating tubules to progress. For
example, whereas in both SHR and Sprague Dawley rats germ cell differentiation reached the spermatocyte stage or beyond in over 90% of tubules, in SHR only 20% of tubules recovered to the late spermatid stage but in Sprague Dawley 42% showed late spermatids.

Although the histological data provide an indication of sperm production, a more quantitative measure is the number of sonication-resistant sperm heads per testis. Irradiated rats showed huge differences between strains, with over 1000-fold differences in testicular sperm production, varying from about $10^2$ in BN and Lewis to almost $2 \times 10^7$ in Sprague Dawley (Fig. 4A). As there were some differences in control values, ranging from $1.4 \times 10^8$ in Lewis to $2.4 \times 10^8$ in Long-Evans, the counts were normalized to the control values (Fig. 4B). BN and Lewis were the most sensitive rat strains with more than a 10,000-fold reduction in sperm production, and Sprague Dawley was most resistant with sperm count remaining at 9% of control. Long-Evans rats were more resistant than Lewis and BN but more sensitive than Wistar Kyoto, Fischer, and SHR; they also showed the largest standard deviation in the counts. These strain differences were consistent with the percentages of tubules with late spermatids in the histological sections (Fig. 3B).

Hormone analyses were performed on one sensitive strain, BN, and one resistant strain, SHR. Serum testosterone, interstitial fluid testosterone, and serum FSH levels in control and irradiated SHR rats were significantly higher than the corresponding values in BN rats, and serum LH levels were significantly higher in control SHR rats than BN rats (Fig. 5). Although there were no significant changes in serum testosterone and LH levels in either BN or SHR rats as a result of irradiation, interstitial fluid testosterone levels were significantly increased after radiation in SHR rats by 1.4-fold, and serum FSH levels significantly increased after radiation in both SHR (1.7-fold) and BN rats (2-fold).

**DISCUSSION**

In this study, we directly compared the recovery of spermatogenesis at 10 weeks after 5 Gy of irradiation in seven different strains or stocks of rats. The results showed that the recovery of spermatogenesis was incomplete in all strains of rats analyzed. Even in the most resistant strain, Sprague Dawley, sperm counts had not even recovered to 10% of control levels.
The contribution of the block in spermatogonial differentiation, previously described in LBNF1 rats, to the failure of recovery was assessed. The atrophic tubules observed in all strains of rats contained similar numbers of type A spermato- gonia (Table 1). These results indicate that the failure of recovery was not due to loss of stem cells but rather to treatment-induced block in the ability of the spermatogonia in these tubules to differentiate and that the major component of the difference in sensitivity between the strains was in the percentage of tubules with evidence of a block in spermato- gonial differentiation at a given dose (Fig. 3). Thus, the radiation-induced block in spermatogonial differentiation is a characteristic of all strains but had not been observed before in Sprague Dawley rats either because the radiation doses were low (Dym and Clermont, 1970; Erickson and Hall, 1983; Huckins, 1978) or the spermatogonia in the atrophic tubules were not noticed in paraffin-embedded tissues (Delic et al., 1987).

Even in the tubules showing differentiated germ cells, there was heterogeneity in the ability to differentiate into the various stages of spermatogenesis. This heterogeneity was observed in all strains, including Sprague Dawley rats, and was not due to loss of stem cells but rather to treatment-induced block in the ability of the spermatogonia in these tubules to differentiate.

**FIG. 2.** Histology of rat testes 10 weeks after irradiation with 5 Gy. (A) BN testis showing atrophic tubules and interstitial edema. (B) The tubules in BN contained mostly Sertoli cells (SC) but some contained a few type A spermato- gonia (Spg). (C) SHR testis showing recovery of spermatogenesis in nearly all tubules. Some tubules in SHR testes (*) showed complete spermatogenesis; other tubules (X) only showed development to the early spermatid stage. (D) Higher magnification image of tubule from irradiated SHR rat showing development to only the early spermatid stage. (Bg) Type B spermatogonia, (P) pachytene spermatocyte, and (RS) round spermatid. Scale (A, C) bar: 100 \( \mu \text{m} \), Scale (B, D) bar: 10 \( \mu \text{m} \).

**FIG. 3.** Recovery of spermatogenesis as measured by the percentage of tubules with morphologically differentiated cells at a specific stage of differentiation or beyond for different strains of rats. (A) TDI defined as differentiation to the B spermatogonial stage or beyond, unless otherwise noted. (B) Percentage of tubules reaching differentiation to specific stages or beyond. The values for groups of irradiated rats with different letters (a, b, and c) are significantly different from each other \( (p < 0.05) \) and groups with the same letter are not.

**TABLE 1**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Spermatogonia per 100 Sertoli cells</th>
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<tbody>
<tr>
<td>BN</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td>Lewis</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>Long-Evans</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>Wistar Kyoto</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>Fischer 344</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>SHR</td>
<td>2.5 ± 0.9</td>
</tr>
<tr>
<td>Sprague Dawley</td>
<td>2.8 ± 0.2</td>
</tr>
</tbody>
</table>

\(^a\)There were insufficient (< 2%) nonrepopulating tubules in 5-Gy irradiated Sprague Dawley rats to perform these counts, so the group irradiated with 6.5 Gy was used for these counts.

\(^b\)No significant differences were observed between different rat strains.
stages (Figs. 2C and 3B). We attribute this to damage to the somatic environment, with some tubules being able to support differentiation to only the spermatocyte or early spermatid stage. Although the present data do not rule out the possibility that this heterogeneity reflects variable delays in initiation of differentiation in various tubules, other data (Kangasniemi et al., 1996) (our unpublished results) show that the differentiation in some tubules does not progress beyond a certain stage even at later postirradiation time points. Because radiation treatment with 5 Gy produced consistent results on the recovery of spermatogenesis within inbred strains of rats but produced differing results between strains, the differences in radiation sensitivity must be attributable to genetic variations between the strains. Consistent with this idea, we found that the standard deviations of the sperm count and tubule differentiation data after irradiation were greater in the outbred Long-Evans rats than in any of the inbred strains, although such a difference was not observed in the outbred Sprague Dawley rats. However, whereas the outbred Sprague Dawley rats were most resistant to radiation effects on spermatogenic recovery, the outbred Long-Evans rats were moderately sensitive, so we cannot conclude that outbred rats are more resistant than inbred ones.

The rat strains were classified according to their differing sensitivities to radiation-induced inhibition of spermatogenic recovery: BN and Lewis were the most sensitive; Long-Evans was intermediate; Wistar Kyoto, Fischer, and SHR were moderately resistant; and Sprague Dawley was most resistant. To investigate a basis for this grouping, we compared the phylogenetic relationships among strains (Saar et al., 2008; Thomas et al., 2003) to resistance levels. The SHR and Wistar Kyoto rats are most closely related and their similar resistance to radiation likely is due to a common set of genes. In contrast, Lewis and Fischer rats, which also are derived from a common ancestor and are relatively closely related, showed a dramatic difference in radiation sensitivity. Lewis are also much more closely related to the more resistant Sprague Dawley rats than they are to the highly sensitive BN strain, which is most genetically distinct of all the rat strains and diverged first in the evolution of strains. Thus, the cause of radiation sensitivity in BN may be different from that in the Lewis rats as it is more likely that two different mutations related to sensitivity would have arisen in the BN and Lewis strains than that mutations to produce resistance arose in all of the five other strains after divergence from the common ancestor with BN. In contrast to the lack of a close relationship between radiation sensitivity and phylogenetic relationship, interstitial fluid accumulation was more closely associated in related strains. The low levels of increase in fluid after irradiation in Lewis, Fischer, Sprague Dawley, and particularly SHR and Wistar Kyoto (Fig. 1D) are consistent with their close phylogenetic relationship; significantly greater increases were observed in Long-Evans and BN, which are more distantly related to the first five strains.

Our results on differential sensitivities of various strains of rats are in general agreement with previous studies using radiation and different toxicant models. The recovery of spermatogenesis after irradiation in Sprague Dawley rats has been shown to be greater than in Wistar rats (Delic et al., 1987). We previously reported that the recovery of spermatogenesis after treatment with the procarbazine was much greater in Sprague Dawley than in Lewis or LBNF1 rats (Parchuri et al., 1993). In addition, Sprague Dawley rats showed greater recovery of spermatogenesis than did Fischer rats after treatment with 2,5-hexanedione, a Sertoli cell toxicant (Blanchard et al., 1996; Boekelheide, 1988; Boekelheide and Hall, 1991). Thus, the interstrain differences appear to be related to the sensitivity to induction of a spermatogonial block after different toxic stresses rather than the sensitivity of the testis to a particular toxicant.

The role of hormones in the strain differences in radiation sensitivity was investigated next. In normal rats, spermatogonial differentiation is qualitatively independent of both testosterone and FSH and occurs even when these hormones are suppressed (Huang and Nieschlag, 1986). However, in
irradiated rats, we demonstrated that the differentiation of type A spermatogonia can be completely inhibited by moderate levels of testosterone alone, independently of the pituitary hormones, or partially inhibited by high levels of FSH (Shetty et al., 2006). In fact, suppression of testosterone for 10 weeks after irradiation of LBNF1 rats (Meistrich et al., 2001) or BN rats (data not shown) can restore the production of differentiated cells in nearly all tubules, as we observed with the more resistant strains without the need for hormonal suppression (Fig. 3A). We therefore tested whether the block in BN rats but not SHR rats could be due to higher levels of testosterone or FSH. To the contrary, there were lower levels of serum and intratesticular testosterone and FSH in BN rats than in SHR rats both before and after irradiation. An alternative hypothesis, that the high levels of testosterone in SHR are responsible for the greater recovery of spermatogenesis, is not consistent with all of our data, as Sprague Dawley rats, the most resistant strain, had levels of testosterone intermediate between the levels in SHR and BN rats (data not shown).

The possible role of the increase in interstitial fluid levels in the inhibition of spermatogonial differentiation was also evaluated because we previously identified a correlation between the two parameters in irradiated LBNF1 rats (Porter et al., 2006). Radiation induced significant increases in interstitial fluid levels in three of the rat strains, most dramatically in BN, a sensitive strain, and Long-Evans, a strain with intermediate sensitivity. In contrast to BN, the other radiation-sensitive strain, Lewis, showed only a small nonsignificant increase in fluid levels. Examination of the relationship between the increase in interstitial fluid and TDI in the various strains (Fig. 6) failed to indicate any significant correlation between the increase in fluid after irradiation and sensitivity of the different strains to the radiation-induced block in spermatogonial differentiation.

The genetic alterations that are responsible for the differences in the recovery of spermatogenesis after radiation in the various strains are not known. The sensitive and resistant strains identified in this study could be used to determine which specific changes in gene expression that occurred after radiation in LBNF1 rats (Zhou et al., 2010) also occur in a sensitive inbred strain identified in this study but not in a resistant strain. In addition, the regions of the genome (quantitative trait loci, QTL) that contain the candidate genes for the interstrain differences in radiation sensitivity can be determined from genetic crosses between strains. Fortunately the BN and SHR rats, a pair of strains for which recombinant inbred rats already available (Tabakoff et al., 2009) showed
A linear regression was performed on the data points and the correlation was against the recovery of spermatogenesis at 10 weeks after 5 Gy of irradiation. Bremner, 1983). In this study, which used only one dose at one which there is eventual recovery of spermatogenesis (Clifton and

large differences in radiation sensitivity, and we are using these strains to identify QTL related to the radiation sensitivity.

The difference between strains in radiation response highlights the importance of knowledge of this information in choosing an animal species and strain within that species for evaluation of risks to human. Blocks in spermatogonial and later germ cell differentiation were observed in all strains and may correspond to the human situation in which no sperm is produced for a prolonged periods after single doses of 1–6 Gy of irradiation to the testis, despite the presence of surviving stem cells from which there is eventual recovery of spermatogenesis (Clifton and Bremner, 1983). In this study, which used only one dose at one time point (5 Gy, 10 weeks), we found very large differences in recovery of differentiation (0–100% of tubules) and sperm production (100-fold differences). It is not known whether there are qualitative differences between strains or only quantitative differences in the dose at which the complete block occurs or differences in the time course of possible subsequent recovery. These questions are being addressed in further experiments.

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Huang, H. F. S., and Nieschlag, E. (1986). Suppression of the intratesticular production (100-fold differences). It is not known whether there are qualitative differences between strains or only quantitative differences in the dose at which the complete block occurs or differences in the time course of possible subsequent recovery. These questions are being addressed in further experiments.

FIG. 6. Radiation-induced increase in testicular interstitial fluid plotted against the recovery of spermatogenesis at 10 weeks after 5 Gy of irradiation. A linear regression was performed on the data points and the correlation was weak ($r^2 = 0.25$) and not statistically significant ($p = 0.25$).


