Comparative Medicine
Copyright 2006
by the American Association for Laboratory Animal Science

Vol 56, No 3
June 2006
Pages 209-214

Damage to Incisors after Nonmyeloablative Total Body Irradiation may Complicate NOD/SCID Models of Hemopoietic Stem Cell Transplantation

Stephen R Larsen,[1] Jenny A Kingham,[1] Marcus D Hayward,[1] and John EJ Rasko[1,2,*]

Immunocompromised murine xenotransplantation models have become an important tool to study stem cell biology. One of the most common recipient strains used is the NOD/SCID mouse, which offers sufficient longevity to quantify moderate levels of engraftment. During pilot experiments, we noted incisor abnormalities 5 to 6 wk after nonmyeloablative doses of irradiation. Here we report a detailed examination of this phenomenon and propose possible explanations and management strategies. A total of 15 NOD/SCID mice received 3 Gy total body irradiation (TBI) and were monitored over 9 wk. A control group of 15 mice were treated in exactly the same way as the study mice except that they did not receive irradiation. A total of 9 TBI mice developed incisor abnormalities between days 40 and 50 after irradiation, resulting in rapid weight loss. No mice in the control group developed incisor abnormalities, however 3 were euthanized prematurely due to the development of thymic lymphoma. Upon development of incisor abnormalities and weight loss, 2 mice in the TBI group had their teeth trimmed and received soft food. Both mice made a rapid recovery and survived for the remainder of the study. The development of incisor abnormalities occurred in 2 substrains, and alterations in antibiotic use and supplementation of the vitamin content of feeds did not prevent the abnormalities. Investigators working with this model should be aware of this complication and modify protocols appropriately.

Abbreviations: NOD, nonobese diabetic; SCID, severe combined immunodeficiency; TBI, total body irradiation

The use of murine xenotransplantation models to study stem cell biology has revolutionized their functional analysis over the last 2 decades.[5] These models facilitate quantitative assessment of human cell biology that cannot otherwise be investigated in vitro. Recipient immunocompromised mouse strains have been used to promote the engraftment of allogeneic or xenogeneic transplanted cells. One of the 1st strains of immunodeficient recipient mice to be used was the severe combined immunodeficiency (SCID) strain.[3] Although human cells could engraft in SCID mice, engraftment occurred very inefficiently due to high natural killer cell activity. A marked improvement in engraftment efficiency occurred with the development of the nonobese diabetic SCID mouse (NOD/SCID strain), originally derived from the SCID strain by Shultz and others at the Jackson laboratory.[12] The NOD/SCID mouse is now the most commonly used strain of immunodeficient mouse for the study of hemopoietic stem cell biology.[8] Engraftment levels in NOD/SCID mice are greater than those in SCID mice because of additional defects in natural killer cell and macrophage function. One drawback to the use of NOD/SCID mice, however, is their shortened lifespan due to the presence of a provirus, *Emv* 30, which is associated with the development of thymic lymphomas.[11] The average lifespan of these mice is approximately 6 mo, but examination of engraftment usually is performed between 6 and 12 wk after transplantation. Whereas it is reasonable to measure engraftment levels after 6 wk, there is some evidence to suggest that donor cells present in the bone marrow of recipient mice at 10 to 12 wk are derived from a more primitive precursor.[2]

Total body irradiation (TBI) of 3 to 3.5 Gy is used to minimize competition from endogenous bone marrow cells and ensure maximum engraftment of donor hemopoietic cells. NOD/SCID mice, like SCID mice, are very susceptible to irradiation because the SCID mutation leads to an inability to repair DNA damage in irradiated cells.[4] Although irradiation doses greater than 10 Gy have been reported to cause dental abnormalities in C57BL/6 mice,[10] there are no reports of these abnormalities in NOD/SCID mice at irradiation levels commonly used for xenotransplantation. During a series of xenogeneic transplantation experiments, we noted the development of brittle teeth in NOD/SCID mice at approximately 5 to 7 weeks after TBI. This abnormality was associated with rapid weight loss in the mice, which usually necessitated culling. Laboratory mice obtain their food by gnawing on rodent pelleted chow with their incisors. Any disruption to incisor function can quickly lead to inanition and subsequent death by starvation unless soft food alternatives are provided. In this report we document the morphologic changes that occur in the incisors of NOD/SCID mice after nonmyeloablative TBI at 3 Gy.

Materials and Methods

Study animals comprised 10- to 12-wk-old specific pathogen-free NOD/SCID (substrain designation, NOD.CB17-Prkdcscid/ARC) female mice were obtained the Animal Resources Centre (Perth, Australia). Mice were housed in a barrier facility in static isolator cages with sterilized paper pelleted bedding ('fibrecycle') and were provided with rodent chow (Irradiated Rodent Diet, Gordon's Stockfeeds, Tillside, Yanderra, Australia) ad libitum

Received: 20 Dec 2005. Revision requested: 23 Mar 2006. Accepted: 25 Mar 2006.
[1]Gene and Stem Cell Therapy Program, Centenary Institute of Cancer Medicine and Cell Biology, University of Sydney, NSW, Australia; [2]Cell & Molecular Therapies, Sydney Cancer Centre, Royal Prince Alfred Hospital, Camperdown, NSW, Australia.
[*]Corresponding author. Email: j.rasko@centenary.usyd.edu.au

Table 1. Scoring of abnormalities in the lower incisors of mice

Score	Description
0	Normal mobility and length; teeth straight
1	Increased mobility, but normal in length and straight
2	Increased mobility, excessive length, and/or crooked
3	One or both incisors broken

and sterile water. This study was approved by the University of Sydney Animals Ethics Committee (approval no., K75/10-2003/3/3822**)** and the Centenary Institute Animal House Management Committee.

A group of 15 mice received 3 Gy TBI from a ^{137}Cs gamma-ray source (Gammacell 40 Exactor, Nordion International, Gammax, Roseville, Australia) at a dose of 92 cGy per min. A control group of 15 age-matched mice were not irradiated but otherwise were treated exactly the same as the study cohort. The irradiated group did not receive a cell transplant; therefore the only difference between the 2 groups was the TBI.

Enrofloxacin (25 mg/ml; approximately 50 mg/kg body weight) was added to the drinking water for all mice, beginning 24 h before TBI and continuing for the duration of the study. Mice were observed daily, and if there was development of hunching or inactivity, they were euthanized if deemed appropriate by animal welfare guidelines. Mice were weighed weekly and were euthanized if they lost more than 20% of their starting body weight. We electively culled 2 control mice and 2 irradiated mice at day 31 of the study to document histologic changes in the apical region of the lower incisors.

The incisors of all mice were photographed with a Leica MZ FLIII microscope (Leica Microsystems Pty Ltd, Gladesville, Australia) to record morphologic changes during the study. On day 64, the day of study completion, the lower incisors were scored, without knowledge of their treatment, according to criteria for mobility, excessive length, and lateral curvature, and whether the teeth were broken (Table 1 and Figure 1). Teeth were classified as having excessive length if they were more than 4.5 mm from the gum line to tip of the erupted tooth. Teeth were classified as crooked if they were not projecting at 90° to the gum line. Mice were not routinely anesthetized for assessment of incisor abnormalities during the experiment because of concerns regarding excessive handling.

Differences in the weight at baseline (day 0) compared with day 42 and the length of teeth were assessed using the Mann–Whitney test (analysis was performed using Graphpad Prism 4, GraphPad Software, San Diego, CA) with statistical significance set at $P < 0.05$. Survival of irradiated mice was analyzed using the Kaplan-Meier survival analysis.

Results

From an initial 30 mice in the study, a total of 12 mice died prematurely or were euthanized before the end of the study. In the control cohort of 15 mice not receiving TBI, 3 mice died prematurely, at days 11, 38, and 61; all 3 were found to have thymic lymphomas on postmortem examination. The remaining control mice were all well and exhibited minimal fluctuation in weight during the study period. Of the 13 mice (excluding the 2 mice electively euthanized at day 31) in the TBI group, 9 were euthanized before the end of the study after developing sudden weight loss, all between days 40 and 50 (Figure 2). The survival of the 2 groups of mice is summarized in Figure 3. The mean weight at baseline was 22.8 g for the control group and 23.5 g for the TBI group ($P = 0.594$). In comparison, after 6 wk (on day 42), the mean weights were 25.2 g for the control group and 20.9 g for the TBI group ($P < 0.005$; Figure 4).

The mean length of incisors was 3.1 mm for the control group and 4.5 mm for the TBI group ($P < 0.005$) at the time of euthanasia. Histologic abnormalities were not detected in the 4 mice that were electively culled at day 31 (2 from each of the control and TBI groups). Postmortem examination of the remainder of the mice in the TBI group did not reveal evidence of sepsis or thymic lymphomas. However, examination of their incisors demonstrated various abnormalities that are summarized in Table 2. In all but 2 mice in the TBI group, at least mild abnormalities were detected. In addition, 4 mice had broken incisors, with 1 mouse having both incisors broken off, and 5 mice had crooked teeth. There was an association between incisor abnormality and early death. All mice with a tooth score of 2 or 3 died prematurely, between days 40 and 51. In contrast, the 2 mice that had scores of 0 were healthy, with normal body weight at the completion of the study.

In an attempt to prevent weight loss and early death, 2 mice underwent trimming and provision of soft feed beginning on day 43, at a time when incisor abnormalities were detected and weight loss had begun. This intervention led to an increase in weight within 2 d after the trimming of teeth. Both of these mice regained weight in excess of their baseline levels and were healthy at the end of the study. On day 64 it was noted that their incisors had regrown to 3 and 4 mm and were normal apart from a mild increase in mobility.

In contrast to the TBI cohort, all mice in the control group that survived to the end of the study were healthy and did not exhibit incisor abnormalities.

Discussion

Xenotransplantation using immunodeficient mouse models plays an important role in the study of stem cell biology. A commonly used mouse is the NOD/SCID strain, mainly because of an increased level of donor cell engraftment compared with that of SCID mice and other strains. Here we report the development of incisor abnormalities in NOD/SCID mice 5 to 7 wk after 3 Gy TBI. There is some evidence that donor hemopoiesis detected at 8 to 12 wk is derived from more primitive precursors; therefore dental abnormalities may compromise longer term experiments designed to study the biology of hemopoietic stem cells.

Dental abnormalities are a well recognized but underappreciated complication of hemopoietic cell transplantation in humans, especially in the pediatric population. Vaughan and others[13] reported the occurrence of dental abnormalities in 99 children who underwent bone marrow transplantation between 1990 and 2000. The frequency of radiographically evident root stunting in permanent teeth increased significantly after bone marrow transplantation, but there was no significant change in the frequency of other dental abnormalities. A different group[6] confirmed that young recipients of hemopoietic stem cell transplants exhibit disturbed dental root growth affecting the development of permanent teeth. This effect occurred in 85% of patients who received TBI, compared with 55% of patients who received chemotherapy without TBI ($P < 0.001$).

Of 13 mice receiving 3 Gy, 9 developed a variety of lower-incisor abnormalities associated with sudden loss of weight, necessitating euthanasia. However, 2 mice with abnormal incisors had their teeth trimmed and were provided with soft feed when a reduction in weight was noted. Within a few days, both mice had regained

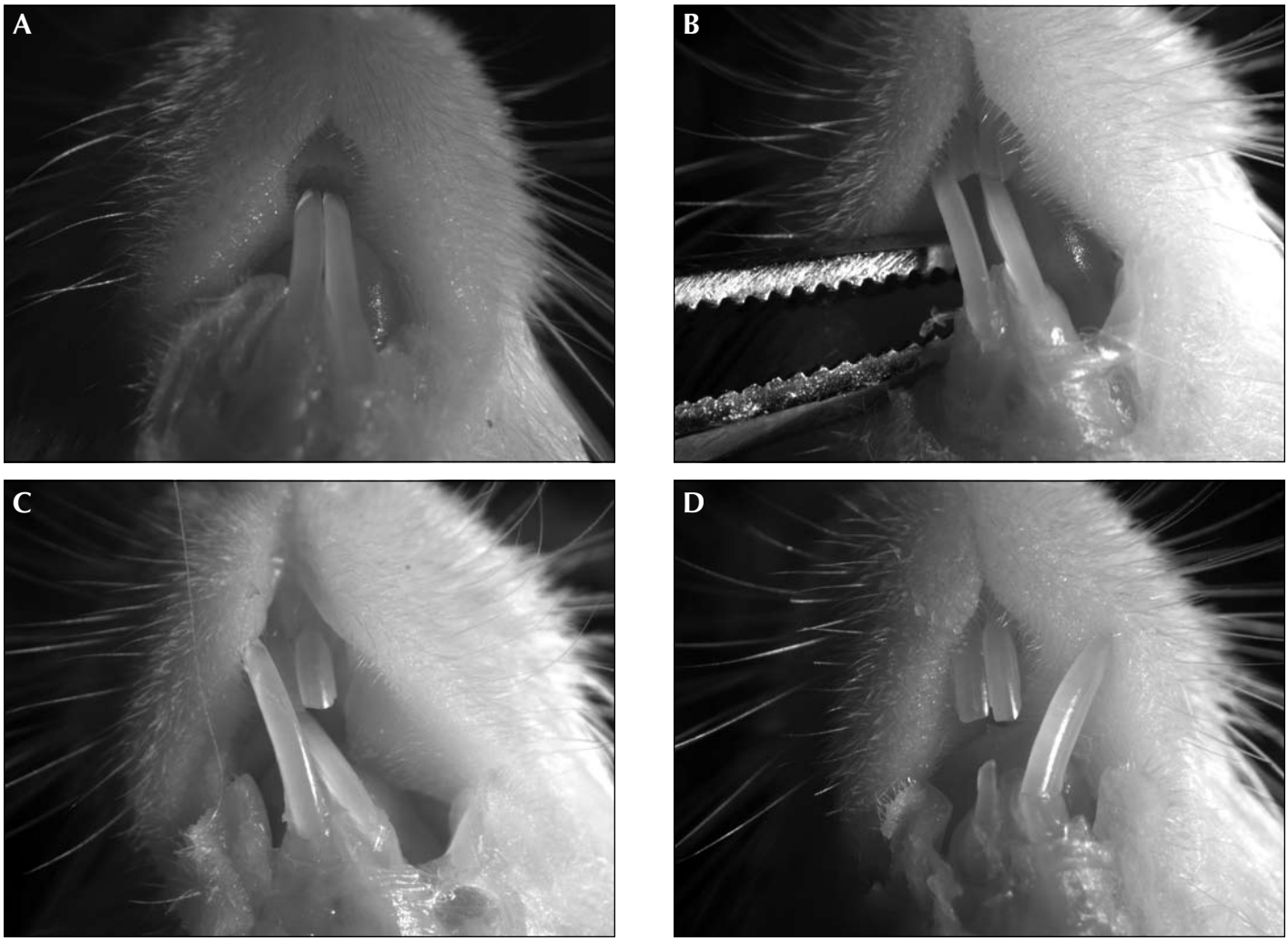

Figure 1. Incisor abnormalities after 3 Gy total body irradiation. Photographs of mice with normal (A), excessively long (B), crooked (C), and broken (D) incisors. The presence and severity of these abnormalities were used to generate the tooth score (Table 1).

weight and were healthy at the termination of the experiment. Although the recovery of weight occurred immediately after this intervention, it is important to emphasize that another 2 irradiated mice recovered spontaneously without teeth trimming; therefore it is difficult to make a definitive conclusion. Nevertheless, the promptness of recovery after trimming suggests that the dental abnormality was the main pathology leading to acute weight loss. Trimming of the teeth most likely improved nutrition by allowing even and solid juxtaposition of upper and lower incisors, which is not present with loose, long, or broken teeth (Figure 1).

Murine dental eruption is dependent on a complex system of tooth progenitor cells, connective tissue, and nutrient vessels. The ameloblasts and odontoblasts initiating enamel and dentine formation, as well as the supportive connective tissue and nutrient, are sensitive to irradiation. The periodontal ligament, responsible for tooth eruption, however, is relatively resistant to irradiation. It is for this reason that teeth continue to erupt after irradiation but that the teeth produced are weakened and loose, leaving them susceptible to fracture and overgrowth. In the case of the 2 mice that received trimming of their teeth, the incisors regrew and were normal in length by the end of the experiment, suggesting that mice recover from the damage to stem cells in the tooth progenitor region when irradiated at 3 Gy. No histologic abnormality was evident between weeks 4 and 5 in 2 mice each from the control and irradiated groups.

Only 2 mice in the TBI group were healthy, with normal incisors and weight, at the completion of the experiment. These mice did exhibit weight loss between days 20 and 40; however they spontaneously recovered. There were no obvious incisor abnormalities in these animals at the end of the experiment, but we cannot exclude transient loosening of the teeth. Damage to upper incisors not observed during this experiment, but damage may occur at higher irradiation doses, as has been noted in a comparison between upper and lower incisor abnormalities in C57BL/6 mice.[10] Other reasons for weight loss include gastrointestinal damage, anorexia, and undetected low-grade sepsis.

Surprisingly, despite widespread use of the irradiated NOD/SCID model for stem cell research, dental abnormalities have not previously been reported. Pearson and others[10] noted similar dental changes in C57BL/6 mice; however, these only occurred after administration of greater than 10 Gy, and teeth abnormalities were noted mainly between 8 and 9 wk postirradiation. A number of laboratories have analyzed NOD/SCID mice 5 to 7 wk after nonmyeloablative TBI,[1,7] raising the possibility that this phenom-

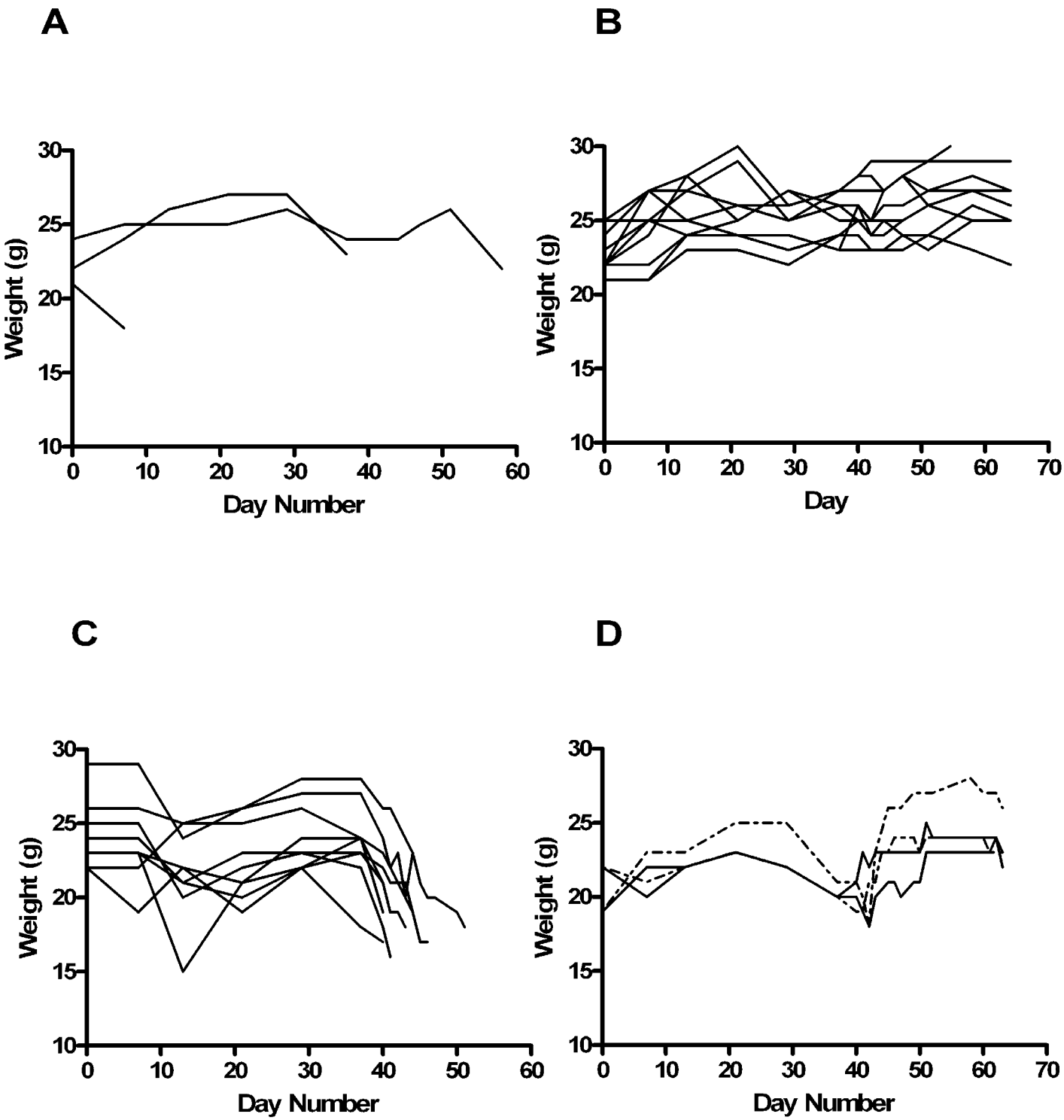

Figure 2. A summary of the weight of mice during the study. Three mice in the control group died prematurely due to the development of thymic lymphomas (A). The remaining mice in the control group maintained a steady weight (B). Of 13 mice in the TBI cohort, 9 lost weight between days 40 and 50 and were euthanized (C). Of the 4 surviving mice in the cohort group (D), 2 had teeth trimming performed at the time of weight loss and increased their weight during the subsequent 2 days (dotted line). The other 2 mice survived without any intervention (solid line).

enon has been seen but not reported. There are several possible explanations for the novelty of our report. First, substrain variability may have resulted in genetic changes rendering the mice more susceptible to irradiation. The 30 mice used, NOD.CB17-*Prkdc*^{scid}/ARC, were the progeny of a 6- rather than 10-generation backcross to the NOD/LtJ strain. However, we have observed identical phenomena in 1 other substrain: NOD.CB17-Prkdc^{scid}/C mice bred at our facility which initially were obtained from the Jackson Laboratories in 2002 as a 10-generation backcross to the NOD/LtJ strain NOD.CB17-Prkdc^{scid}/J. Therefore, we have dem-

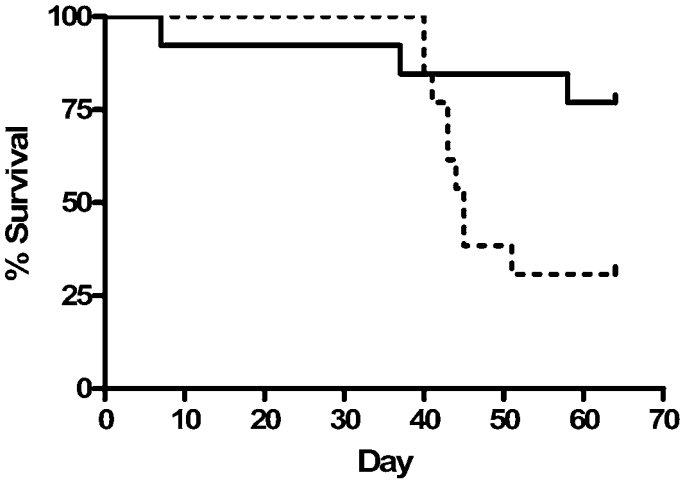

Figure 3. Kaplan–Meier survival curves of control and irradiated mice that died prematurely or remained alive until the end of the study, excluding those that were electively euthanized for histologic examination on day 31.

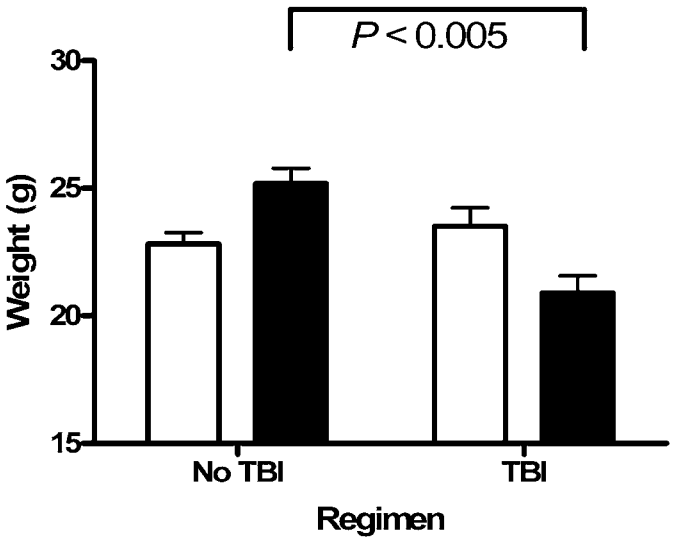

Figure 4. Weight of mice (mean ± 1 standard deviation) at baseline (day 0; solid bars) and day 42 (open bars). There was no difference in the weight at baseline between control and TBI cohorts. By day 42, there was a significant ($P < 0.005$) difference in the respective mean weights (control, 25.2 g; TBI, 20.9 g).

onstrated incisor abnormalities in 2 substrains.

A second possibility to explain the novelty of our report of incisor abnormalities in irradiated NOD/SCID mice relates to the irradiator used. We used gamma irradiation from a ^{137}Cs source, and it is possible that the phenomena observed are specific to this source. However, this irradiator is used to administer TBI to hundreds of mice of different background strains every year, usually at much higher doses, and incisor abnormalities have not been noted. The machine has been calibrated twice over the last 2 years without changes in dental abnormalities in irradiated NOD/SCID mice being noted. A potential solution to prevent the development of incisor abnormalities caused by TBI is to shield the heads by using a custom-made lead cone. We are in the process of investigating this adaptation further.

We also examined other variables such as nutrient levels and antibiotic use and have not found either to be contributing factors. During earlier experiments, we obtained dietary analysis of the irradiated feed and discovered that the feed was low in vitamins K and B6, when compared with levels recommended in the National Research Council's *Nutrient Requirements of Laboratory Animals*.[9] Correction of these abnormalities did not affect the incidence or severity of dental abnormalities. The absolute levels and ratio of calcium and phosphorus and the level of vitamin D were also within approved guidelines. Altering the antibiotics used to neomycin with polymyxin B (3.75 and 1.95 mg/kg, respectively, daily) or to sulfamethoxazole with trimethoprim (12 and 24 mg/kg, respectively, daily) in drinking water did not affect the development of incisor abnormalities after TBI. Altering the duration of enrofloxacin administration from ongoing administration in drinking water to administration for 3 wk only postirradiation also was tested and showed no substantial benefit.

In conclusion, we have examined the development of lower-incisor abnormalities in NOD/SCID mice 5 to 7 wk after 3 Gy TBI. Investigators using this strain to study the biology of hemopoietic stem cells should be aware of the possibility of this phenomenon when using this model. Intervention with teeth trimming and the provision of soft feed at the 1st sign of weight loss may prolong the lifespan of these mice, allowing analysis at a later time point.

Acknowledgments

During these studies, Stephen Larsen received support from the Anthony Rothe Trust, Royal Australian College of Physicians, and the Cancer Institute NSW. We acknowledge grant support from the Leukaemia Foundation and National Health and Medical Research Council of Australia.

References

1. **Astori G, Adami V, Mambrini G, Bigi L, Cilli M, Facchini A, Falasca E, Malangone W, Panzani I, Degrassi A.** 2005. Evaluation of ex vivo expansion and engraftment in NOD-SCID mice of umbilical cord blood CD34+ cells using the DIDECO "Pluricell System". Bone Marrow Transplant **35:**1101–1106.
2. **Cashman JD, Lapidot T, Wang JC, Doedens M, Shultz LD, Lansdorp P, Dick JE, Eaves CJ.** 1997. Kinetic evidence of the regeneration of multilineage hematopoiesis from primitive cells in normal human bone marrow transplanted into immunodeficient mice. Blood **89:**4307–4316.
3. **Custer RP, Bosma GC, Bosma MJ.** 1985. Severe combined immunodeficiency (SCID) in the mouse. Pathology, reconstitution, neoplasms. Am J Pathol **120:**464–477.
4. **Fulop GM, Phillips RA.** 1990. The scid mutation in mice causes a general defect in DNA repair. Nature **347:**479–482.
5. **Guasch G, Fuchs E.** 2005. Mice in the world of stem cell biology. Nat Genet **37:**1201–1206.
6. **Holtta P, Hovi L, Saarinen-Pihkala UM, Peltola J, Alaluusua S.** 2005. Disturbed root development of permanent teeth after pediatric stem cell transplantation. Dental root development after SCT. Cancer **103:**1484–1493.
7. **McKenzie JL, Gan OI, Doedens M, Dick JE.** 2005. Human short-term repopulating stem cells are efficiently detected following intrafemoral transplantation into NOD/SCID recipients depleted of CD122+ cells. Blood **106:**1259–1261.
8. **Meyerrose TE, Herrbrich P, Hess DA, Nolta JA.** 2003. Immune-deficient mouse models for analysis of human stem cells. Biotechniques **35:**1262–1272.
9. **National Research Council**. 1995. Nutrient requirements of laboratory animals. 4th ed. Washington (DC): National Academy Press.

Table 2. Changes in lower incisors of control and irradiated mice

Mouse identifier	Mobility score[a]	Shape	Teeth trimmed?	Length of teeth (mm)	Day culled	Tooth score[b]
Control mice[c]						
C1	1	Straight	No	3.5	64	0
C2	1	Straight	No	4	64	0
C3	1	Straight	No	3	64	0
C4	1	Straight	No	3	64	0
C5	1	Straight	No	3	64	0
C6	1	Straight	No	3.5	64	0
C7	1	Straight	No	3	38	0
C8	1	Straight	No	3	64	0
C9	1	Straight	No	3	64	0
C10	1	Straight	No	2.5	64	0
C11	1	Straight	No	3	64	0
C12	1	Straight	No	not applicable	7	0
C13	1	Straight	No	not applicable	58	0
		Mean ± 1 SD		3.1 ± 0.1	57 ± 4.6	0 ± 0
Irradiated mice[d]						
S1	2	Straight	Yes	3	64	1
S2	2	Straight	Yes	4	64	1
S3	2	Crooked	No	5	40	2
S4[e]	not scored	Broken ×2	No	not applicable	41	3
S5	2	Crooked	No	5	45	2
S6	3	Broken ×1	No	5	51	3
S7	3	Broken ×1	No	5.5	45	3
S8	1	Straight	No	3	64	0
S9	3	Straight	No	5	43	2
S10	3	Crooked	No	4	40	2
S11	3	Broken ×1	No	5	43	3
S12	1	Straight	No	3	64	0
S13	3	Crooked	No	5	43	2
		Mean ± 1 SD		4.6 ± 0.3	47 ± 2.7	2 ± 0.3

[a]Mobility score: range of 1 to 3. A score of 1 indicates almost no movement, and 3 indicates marked movement.
[b]Tooth score: see Table 1 for details.
[c]Control mice do not include the 2 mice euthanized on day 31 for histopathology or the mice removed from the study due to thymic lymphoma.
[d]Study mice do not include the 2 mice sacrificed at day 31 for histopathology.
[e]Because both teeth were broken close to the gum, the mobility and length were not recorded.

10. **Pearson AE, Phelps TA.** 1981. Radiation effects on mouse incisor teeth following whole-body doses of up to 16 gray. Int J Radiat Biol Relat Stud Phys Chem Med **39:**409–417.
11. **Prochazka M, Gaskins HR, Shultz LD, Leiter EH.** 1992. The non-obese diabetic scid mouse: model for spontaneous thymomagenesis associated with immunodeficiency. Proc Natl Acad Sci U S A **89:**3290–3294.
12. **Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Tennent B, McKenna S, Mobraaten L, Rajan TV, Greiner DL.** 1995. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. J Immunol **154:**180–191.
13. **Vaughan MD, Rowland CC, Tong X, Srivastava DK, Hale GA, Rochester R, Kaste SC.** 2005. Dental abnormalities after pediatric bone marrow transplantation. Bone Marrow Transplant **36:**725–729.