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Late complications following total-body irradiation and bone marrow rescue in mice: predominance of glomerular nephropathy and hemolytic anemia

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Late mortality and pathology were assessed in various mouse strains following total-body irradiation (TBI) and bone marrow transplantation. A, C57BL/6, B6AF$_1$, LP and C3H mice received TBI in two fractions 3 h apart at total doses of between 11 and 15 Gy. They were then transplanted with syngeneic bone marrow cells providing sufficient reconstitution to avoid hemopoietic failure. Long-term survival data revealed both radiation dose- and strain-dependent onset of mortality between 1 and 2 years post-treatment. Renal damage appeared to have contributed to the late mortality in most treatment groups as shown by glomerular lesions, elevated blood urea nitrogen and an accompanying fall in hematocrit. Hemolysis was deduced to be the major cause of anemia, as concluded from results of ^{51}Cr-labeled erythrocyte survival. No decrease in erythropoiesis was evident as seen from spleen and bone marrow ^{59}Fe uptake. These findings are together consistent with the manifestation of a hemolytic uremic syndrome (HUS) with kidney glomeruli representing the principal sites of injury responsible for both renal dysfunction and microangiopathic hemolysis.

1. Introduction

It is now well established that hemopoietic failure following total-body irradiation (TBI) can be avoided by bone marrow transplantation (BMT), enabling the host to withstand higher radiation doses without acute lethality. Considerable interest has focused on this technique since it constitutes an effective therapeutic strategy for treating hemopoietic disorders and other disseminated disease. Lung complications now represent a major dose-limiting clinical concern (Keane *et al.* 1981), prompting a search for ways of improving the therapeutic benefit by modifying TBI conditioning (Goolden *et al.* 1983, Thomas *et al.* 1982). However, beyond the period dominated by pulmonary toxicity, little is known regarding the long-term consequences of changing BMT preparation. We were therefore interested in evaluating and characterizing the delayed effects of TBI in a murine model of syngeneic BMT.

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In these studies TBI doses of 11–15 Gy delivered in two fractions were selected because preliminary experiments had revealed no splenic endocolonies or animal survival without marrow transplantation at doses above 10 Gy. In addition, complete engraftment following 11 Gy was commonly observed in recipients of 10^7 bone marrow cells from allogeneic (H-2 compatible) or semi-allogeneic donors (Mauch *et al.* 1985, Ferrara *et al.* 1988). In both marrow transplantation and toxicity experiments, research groups tend to concentrate their investigations on one particular animal strain, making it difficult to compare data directly between institutions. Furthermore, knowledge of any genetic diversity can provide useful information towards resolving the mechanism(s) of radiation-induced tissue injury. Our TBI studies therefore encompassed five different mouse strains, which were monitored for survival and underlying pathology. The recent clinical reports of late renal damage and hemolytic uremia in BMT patients receiving TBI (Antignac *et al.* 1989, Bergstein *et al.* 1986, Chappell *et al.* 1988, Tarbell *et al.* 1988) have focused our attention on radiation nephropathy and its relation to anemia in these animals. The results presented offer valuable insights regarding the relative importance of kidney glomeruli and tubules as critical sites for late radiation injury.

2. Materials and methods

2.1. *Treatment*

Male mice of A/J, C57BL/6J, C57BL/6♀ × A♂ F_1 (B6AF$_1$), LP/J and C3H/HeJ strains were obtained from the Jackson Laboratory, Bar Harbor, ME. In earlier experiments mice were maintained in a conventional facility of unknown pathological status. Later experiments on C3H mice were barrier-maintained in isolator cages and were free of known viral pathogens. At 12–14 weeks of age they were irradiated to the whole body using ^{137}Cs through opposing portals at 100–108 cGy/min. Radiation doses were delivered in two fractions separated by 3 h followed by the intravenous infusion of 10^7 bone marrow cells freshly prepared from the hind limbs (femur and tibia) of syngeneic donors.

2.2. *Blood analysis*

Blood samples (20 μl) were collected from the tail tip in heparinized capillary tubes and centrifuged for hematocrit (HCT) determination. Sera above the buffy coat were stored at −20°C for subsequent blood urea nitrogen (BUN) assay using Sigma diagnostic kit 536-B. Serum (5 μl) was added to 1 ml of fresh BUN end-point reagent, mixed and allowed to stand for 15 min at room temperature. Samples were read against a reagent-only blank at 340 nm.

2.3. *Autopsy*

Randomly allocated groups of mice were sacrificed by CO_2 asphyxiation, the thorax opened and the pleural fluid absorbed on tissue paper and weighed. The lungs were inflated with 10 per cent neutral buffered formalin and, together with both kidneys, immersed in fixative. Tissue samples were dehydrated, embedded in paraffin or methacrylate, sectioned at 2 μm and stained with hematoxylin and eosin or silver methenamine.

At 38 and 68 (see below) weeks after 12·5 Gy TBI, the kidneys of five control and five treated C3H mice were separately prepared for transmission electron microscopy. Here the kidneys were fixed for 4 h in cold 3 per cent glutaraldehyde in 0·1 M cacodylate buffer. Cubes (1 mm) were dissected from the kidney cortex,

rinsed in cacodylate buffer and then post-fixed in cold 1 per cent oxmium tetroxide for 2 h. The samples were dehydrated through graded ethanol and propylene oxide and embedded in Epon. Thick (1·0 μm) sections were cut and stained with toluidine blue to select areas for thin sectioning. Thin (0·06–0·08 μm) sections were then cut, stained with uranyl acetate followed by lead citrate and examined on a Zeiss EM-109 electron microscope.

On the day before sacrifice, $B6AF_1$ and C3H mice were monitored for resting breathing rate (BR) using a total body plethysmograph described by Travis *et al.* (1979).

Animals observed to be moribund and hypothermic were analysed for HCT and BUN then euthanized and subjected to the same autopsy procedure.

2.4. *Red cell survival and erythropoiesis* in vivo

At 65 weeks after 12·5 Gy TBI and BMT, groups of five control and treated C3H mice were selected, on the basis of prior HCT measurements, for estimating erythrocyte survival and radioiron uptake.

An untreated C3H mouse was used as a syngeneic donor of ^{51}Cr-tagged red blood cells (RBC). Blood (1 ml) was withdrawn from the thoracic cavity in a heparinized pipette and washed with 3 ml Hanks balanced salt solution (HBSS) containing 5 per cent fetal calf serum (FCS). The red cell pellet was resuspended in 2 ml HBSS/5 per cent FCS containing 7·4 MBq Na_2 [^{51}Cr]O_4 and the suspension incubated at 37°C for 30 min in a shaking water bath. Three millilitres of HBSS/FCS were then added and the red cells pelleted by centrifugation as before. The pellet was washed twice in HBSS/FCS and then finally resuspended in 3 ml HBSS, kept on ice and 0·2 ml injected i.v. Blood samples (20 μl) were collected from the tail tip in heparinized hematocrit tubes on days 1, 8, 14 and 21 after [^{51}Cr]RBC injection. The tubes were centrifuged, the HCT determined and the packed red cells isolated and blown into 0·5 ml distilled water and stored at −20°C for subsequent ^{51}Cr counts on a gamma counter. All samples were counted on the day after sacrifice and expressed as a percentage of the Day 1 count.

The uptake of ^{59}Fe during hemoglobin synthesis was used as an *in vivo* measure of erythropoiesis. An injection of 0·04 MBq $^{59}FeCl_2$ was made i.p. at 19 days after [^{51}Cr]RBC administration. Two days later the mice were bled for HCT, BUN, white cell count and new methylene stained smears. The mice were then sacrificed by CO_2 asphyxiation and the pleural fluid, lungs, spleen and kidneys were weighed and tissues placed in 10 per cent NBF. The fixed spleens and femurs were gamma counted for ^{59}Fe radioactivity and, together with lungs and one kidney, processed for methacrylate embedding and light microscopy. The other kidney was fixed in 3 per cent glutaraldehyde and prepared for electron microscopy as described above.

3. Results

3.1. *Survival*

Hemopoietic reconstitution by infusion of bone marrow cells prevented acute marrow failure in most animals (>90 per cent) throughout the first month after TBI. Survival persisted during the first year, after which it fell depending on radiation dose and genetic strain as shown from median survival time data in table 1. Life-shortening induced by TBI appeared to be related to the normal life expectancy for each mouse strain, occurring earlier in A and LP mice. C57BL mice showed the longest survival times, a genetic trait that appeared to dominate in the $B6AF_1$ hybrids.

Table 1. Median survival times (MST) after TBI and BMT for each mouse strain and dose group.

Strain	TBI dose (Gy)	MST (weeks)	95 per cent CI†
A	0	92	80–100
	11	68	60–72
	12	60	60–64
	13	40	40–56
LP	0	104	96–112
	11	56	56–68
C3H	0	112	108–116
	12·5	80	64–84
B6	0	>124	
	11	96	92–100
$B6AF_1$	0	>104	
	11	96	72–106
	13	76	72–80
	15	56	52–68

† Non-parametric confidence intervals (CI) were calculated according to Brookmeyer and Crowley (1982).

3.2. *Pathology in C3H mice*

Sequential blood analyses and kidney weight measurements were performed in C3H mice receiving 12·5 Gy TBI. Pooled from four separate experiments, figure 1 displays the time course of these data against survival. This shows a rise in BUN levels, a fall in hematocrit and a decrease in kidney mass concomitant with the period of mortality.

As shown in figure 2 and table 2, during the period of 65–86 weeks post-treatment the loss of ^{51}Cr-tagged erythrocytes in the irradiated mice was significantly greater than that seen in control mice, indicating a hemolytic process. Residual erythropoiesis as measured by ^{59}Fe uptake in whole spleens and femurs was, on the other hand, similar in control and irradiated mice (table 3). The spleens of irradiated mice were, however, of lower mass than controls; hence the amount of ^{59}Fe uptake per unit spleen weight was significantly higher in treated animals. By 2 days after ^{59}Fe injection, most of the labeled cohort of hemoglobin-synthesizing erythroid cells (erythroblasts and reticulocytes) will have passed into the circulation (Papayannopoulou and Finch 1975). This is shown from the present results in table 3 as a high ^{59}Fe activity in peripheral erythrocytes, which was significantly higher in treated mice. Other measurements at autopsy of these animals are compared in table 4 to show a significant decrease in kidney weight and HCT and a rise in BUN and reticulocyte index (RI). Pleural fluid levels were also significantly high in irradiated mice compared with controls, but since the breathing rates were normal this increase was unlikely to have clinical consequences. Lung weights and white cell counts were within the normal range.

The autopsy sections from mice scarificed at 38 weeks after TBI revealed pathology confined essentially to the kidney. Light microscopy showed necrosis of glomerular capillary tufts with plugging of erythrocytes (figure 3). Electron microscopy of these kidneys confirmed the light microscopic observations and

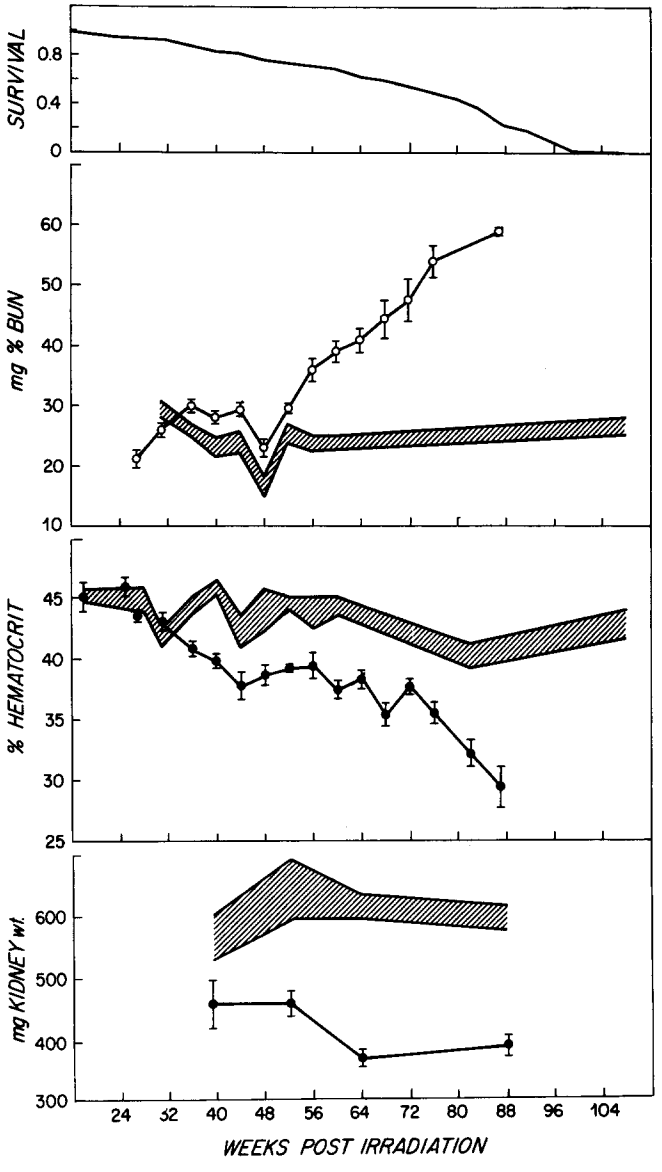

Figure 1. Survival (total of 71 treated mice), blood urea nitrogen (BUN) levels (5–28 mice per point), blood hematocrit (5–28 mice per point) and kidney mass (5–6 mice per point) as a function of time from 12·5 Gy TBI and 10^7 syngeneic bone marrow cells in C3H mice. Shaded area and error bars represent ± 1 SEM for control and irradiated mice, respectively.

showed complete segmental necrosis of glomeruli involving mesangial, endothelial and epithelial cells. Capillary lumina were either obliterated or filled with trapped erythrocytes. The renal tubules displayed no histological abnormalities.

More widespread and severe renal damage was seen at 68 weeks with both chronic and on-going acute glomerular disease. Many capillary tufts were acellular (figure 4, inset). Glomerular crescent formation and sclerosis were frequent.

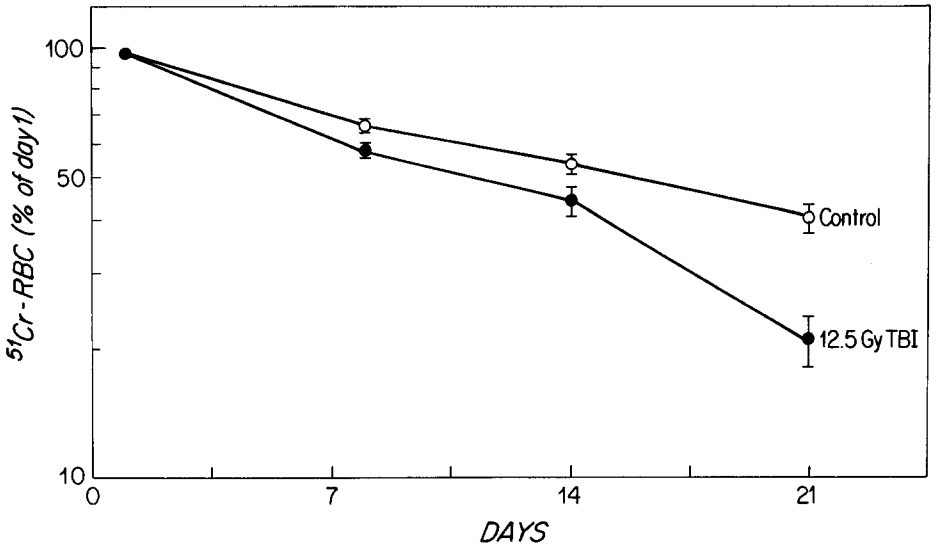

Figure 2. Survival of ^{51}Cr-labeled red blood cells in control (○) and 12·5 Gy irradiated mice (●) over 65–68 weeks after TBI. Results are expressed as a percentage of the Day 1 radioactive count and presented as mean ± 1 SEM.

Mesangial cells were increased and there was increased basal lamina material. Electron microscopy revealed areas of reduplicated basal lamina. Some tufts were acellular and filled with necrotic debris, fibrin and collagen. Many capillaries were occluded by platelets and trapped erythrocytes (figure 4). Capillary damage was often accompanied by fusion of epithelial foot processes. The proximal and distal tubules were morphologically unremarkable.

Sections of other organs showed no specific pathology. The lungs from treated mice exhibited only minor changes consisting of very occasional lymphocytic peribronchial cuffing and focal accumulation of foamy alveolar macrophages. The femoral bone marrow showed normal erythroid cellularity and the spleen, except for its smaller size (table 3), was unremarkable.

Table 2. Parameters for [^{51}Cr]RBC survival at 65–68 weeks after TBI (12·5 Gy) and BMT in C3H mice (mean ± 1 SEM).

Treatment	Rate constant α (days^{-1})	Half-life $t_{1/2}$ (days)	Mean life-span (days)
Controls	0·047	15·4	22·3
(n=5)	0·004	1·4	2·0
12·5 Gy TBI	0·070†	10·0†	14·5†
(n=5)	0·005	0·7	1·0

The monoexponential equation $C = A^{-\alpha t}$ (where α = rate constant) was fitted to the [^{51}Cr]RBC data from individual mice using a non-linear least-squares computer program. Values from treated mice were compared with corresponding controls using the Mann–Whitney U-test. †$p < 0{\cdot}05$.

Table 3. ^{59}Fe uptake in spleen, femur and blood at 68 weeks after TBI (12·5 Gy) and BMT in C3H mice (mean ± 1 SEM).

Treatment	Spleen weight (mg)	^{59}Fe per spleen†	^{59}Fe per g spleen	^{59}Fe per femur	^{59}Fe per ml blood
Controls	98·6	1·15	11·6	0·242	11·8
($n=5$)	3·7	0·09	0·7	0·019	0·7
12·5 Gy TBI	54·7†	1·20^{NS}	21·7†	0·308^{NS}	17·7†
($n=5$)	3·4	0·12	1·1	0·037	0·7

† Two-day percentage uptake of radioiron.

Measurements from untreated mice were compared with corresponding controls using the Mann–Whitney U-test. †$p<0·05$; ^{NS} = no significant difference ($p>0·05$).

3.3. *Pathology in A, B6 and B6AF$_1$ mice*

Uremia and anemia were also seen in groups of irradiated A mice at 52 and 60 weeks from transplant (table 5). Four A mice were autopsied when sick between 41 and 54 weeks after 11 or 13 Gy. Blood analyses showed very low HCT (6–19 per cent) and high BUN (33–60 mg per cent) while their kidneys were pale and shrunken (201–356 mg). Pleural fluid levels were slightly above normal (0·04–0·11 ml) and their lungs were of normal histology and weight (126–138 mg).

A different pathological picture emerged in groups of B6AF$_1$ mice sacrificed at 62 weeks (table 6). Large pleural fluid volumes accounted for rapid breathing rates in irradiated animals while HCT, BUN and lung weight values were near normal. The kidney weights were, however, significantly below the controls. At the later time of 76 weeks, pleural effusions and lung dysfunction no longer appeared to be a problem, though anemia and uremia was clearly evident. Six B6AF$_1$ mice were autopsied when sick. Three received the highest dose of 15 Gy 58–64 weeks previously, and had large pleural effusions of above 1 ml (1·4–2·0 ml) with HCT, BUN and lung weights within the normal range (46–50 per cent, 15·5–20 mg per cent and 170–227 mg, respectively). The other three sick B6AF$_1$ mice received a lower dose of 13 Gy 69–75 weeks beforehand, and had no clinically significant

Table 4. Measurements on autopsied C3H mice at 68 weeks after TBI (12·5 Gy) and BMT (mean ± 1 SEM).

Treatment (no. mice)	Body weight (g)	HCT (%)	BUN (mg%)	RI (%)	WBC (10^7/ml)	Kidney weight (mg)	Lung weight (mg)	Pleural fluid (mg)	BR (Br/min)
Controls	33·0	44·3	22·3	1·25	1·27	714	179	16·4	262
($n=5$)	1·4	1·1	1·6	0·10	0·08	30	6	2·1	17
12·5 Gy	24·6†	35·2†	44·8‡	2·10†	1·34^{NS}	461†	174^{NS}	75†	245^{NS}
($n=5$)	0·6	1·6	2·3	0·13	0·16	17	9	18	4

All measurements from treated mice were compared with corresponding controls using the Mann–Whitney U-test.

†$p<0·05$; ‡$p<0·01$; ^{NS} = no significant difference ($p>0·05$).

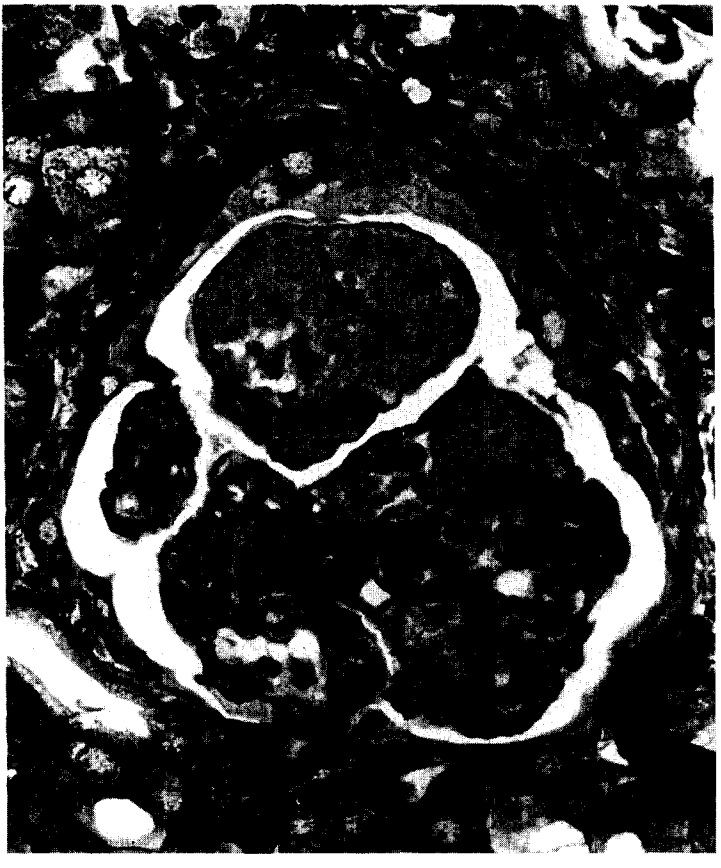

Figure 3. Glomerulus on light microscopy at 38 weeks after 12·5 Gy TBI in a C3H mouse. There is segmental necrosis of the glomerulus with trapping of red cells (silver methenamine, × 600).

pleural effusions (0·06–0·4 ml) but were anemic (HCT = 38–40 per cent) and uremic (BUN = 40–63 mg per cent).

Of the kidney specimens taken from A, B6 and $B6AF_1$ mouse strains between 1 and 2 years after TBI, all exhibited progressive glomerular damage, ranging from acute mesangiolysis and crescentic fibrin deposition to chronic degenerative sclerosis. Proximal or distal tubular alterations were absent or minimal as compared with concurrent unirradiated controls.

4. Discussion

A delayed manifestation of renal dysfunction and anemia became the striking feature that appeared to limit survival following BMT in various mouse strains at TBI doses of 11–13 Gy. A higher dose of 15 Gy was delivered to $B6AF_1$ mice and here an earlier incidence of pleural effusions replaced kidney damage as the major problem affecting survival. These effusions, while often confused with pulmonary fibrosis, are commonly encountered as a late and lethal complication of localized thoracic irradiation (Down 1986, Down *et al.* 1988). It is therefore conceivable that the effusions seen after TBI in the present study represent the same phenomenon.

Figure 4. Glomerulus on light (inset) and electron microscopy at 68 weeks after 12·5 Gy. By light microscopy there are acellular tufts (arrow) as well as a dramatic increase in mesangial matrix (silver stain, ×400). The electron micrograph shows capillary tufts with accumulation of necrotic debris, platelets, collagen and deposits that resemble fibrin. There are also trapped erythrocytes (arrow), thickened basement lamina and fusion of epithelial foot processes (×9000).

The low radiation tolerance of the kidneys is consistent with earlier reports obtained from local bilateral irradiations and TBI on mice (Glatstein *et al.* 1977, Stewart *et al.* 1984, Cosgrove *et al.* 1964, Covelli *et al.* 1974, Travis *et al.* 1985) and on rats (Moulder *et al.* 1987a).

An inverse relationship between the time to injury and radiation dose delivered is typical of late-reacting tissues in general (Michalowski 1981) and is well described for kidney (Lebesque *et al.* 1986). The earlier incidence of mortality in A and LP mice may be attributed to a higher overall sensitivity of the kidney towards radiation. Alternatively, the turnover time of the target cell population may be shorter in these strains as compared with C57BL or $B6AF_1$ mice with a consequent earlier expression of radiation damage. Large variations in response time among different mouse strains have also been addressed for radiation injury to the lung (Down and Steel 1983, Down *et al.* 1986) and provides an invaluable approach for

Table 5. Peripheral hematocrit (HCT) and blood urea nitrogen (BUN) measurements on A mice at 52 and 60 weeks after TBI and BMT (mean ± SEM).

TBI dose (Gy) (no. mice)	HCT (%)	BUN (mg %)
52 weeks		
0 ($n=6$)	48·7 0·5	21·0 1·4
11 ($n=16$)	45·2^{NS} 1·2	28·5‡ 1·2
13 ($n=4$)	37·0† 5·0	27·3^{NS} 4·0
60 weeks		
0 ($n=6$)	45·5 1·0	18·6 0·8
11 ($n=10$)	40·8† 1·3	30·4‡ 3·6

All measurements from treated mice were compared with the corresponding controls using the Mann–Whitney U-test. †$p<0·05$; ‡$p<0·01$; ^{NS}=no significant difference $p>0·05$).

Table 6. Measurements on five autopsied B6AF$_1$ mice at 62 and 76 weeks after TBI and BMT (mean ± 1 SEM).

TBI dose (Gy) (no. mice)	HCT (%)	BUN (mg%)	Kidney weight (mg)	Lung weight (mg)	Pleural fluid (ml)	BR (Br/min)
62 weeks						
0 ($n=5$)	45·8 0·5	22·8 1·0	524 13	195 6	0·02 0·002	301 8
11 ($n=5$)	46·0^{NS} 0·9	21·9^{NS} 1·9	416‡ 7	175† 3	0·04† 0·005	338† 5
13 ($n=5$)	40·4^{NS} 3·1	24·9^{NS} 0·9	395‡ 15	173^{NS} 7	0·35† 0·24	350† 13
15 ($n=5$)	44·0^{NS} 1·3	24·5^{NS} 3·1	403‡ 12	213^{NS} 25	0·30‡ 0·14	369† 30
76 weeks						
0 ($n=5$)	49·2 0·5	21·5 1·5	637 22	232 14	0·04 0·01	300 5
13 ($n=5$)	39·2† 2·6	31·3† 3·1	482† 44	236^{NS} 15	0·16† 0·04	308^{NS} 6

All measurements from treated mice were compared with the corresponding controls using the Mann–Whitney *U*-test. †$p<0·5$; ‡$p<0·01$; ^{NS}=no significant difference ($p>0·05$).

further elucidating the pathogenesis of radiation damage in tissue systems of slow cell renewal.

Identifying which of the kidney cell types are the most important for initiating radiation injury is currently a topic of much debate. Our observed predominance of glomerular lesions with minimal effects on kidney tubules agrees with the detailed sequential histological study of Glatstein *et al.* (1977) in the irradiated kidneys of C3H mice. Similar observations have been documented following experimental TBI in mice (Guttman and Kohn 1963, Covelli *et al.* 1974, Down *et al.* 1989) and in hamsters (Kohn and Guttman 1964). In contrast, tubular degeneration in the absence of glomerular injury has been described by others for irradiated mouse kidney (Travis *et al.* 1985, Withers *et al.* 1986, Michalowski *et al.* 1986). These apparent inconsistencies may be due to strain differences, but in our study glomerular damage was found to be present among all four mouse strains investigated with histology. Clearly more studies are needed to resolve these discrepancies. Nevertheless, it is important to compare these experimental observations with the pathology of radiation nephropathy in humans. Here, microscopic evaluation points toward the glomerulus as the most susceptible (Zuelzer *et al.* 1950, Rosen *et al.* 1964, Keane *et al.* 1976, Fajardo 1982, Steele and Lirenman 1979, Bergstein *et al.* 1986, Chappell *et al.* 1988, Tarbell *et al.* 1988).

In clinical cases renal dysfunction is often also accompanied by hemolytic anemia, either following local abdominal radiotherapy (Steele and Lirenman 1979) or TBI (Antignac *et al.* 1989, Bergstein *et al.* 1986. Chappell *et al.* 1988, Marshall and Sweny 1986, Tarbell *et al.* 1988). Our own observations of anemia with reduced erythrocyte life-span in C3H mice support hemolysis, as does our earlier report of fragmented erythrocytes and reticulocytosis during renal failure in B6 mice at 95 weeks after fractionated TBI (Down *et al.* 1989). A close relationship between the development of uremia and anemia in C3H mice suggests a common pathological etiology. Indeed, radiation dose–response curves for hematocrit have yielded estimations similar to renal clearance for assessing radiation kidney tolerance, even under conditions of large dose-sparing with multiple fractionated irradiation (Stewart *et al.* 1984). The electron microscopic evluation of the irradiated kidney has allowed us to identify marked disruption of the capillary tufts within glomeruli with features that compare very well with other EM studies on mice (Fajardo *et al.* 1976) and humans (Rosen *et al.* 1974, Keane *et al.* 1976, Chappell *et al.* 1988). This may therefore be the critical site responsible for initiating both renal dysfunction and a microangiopathic hemolysis. A reduced production of erythropoietin by kidneys has been used as an alternative explanation for reduced hematocrits during radiation nephropathy (Alpen and Stewart 1984, Fisher *et al.* 1964, Robbins *et al.* 1989). However, erythropoiesis *in vivo* as measured by radioiron uptake in erythroid precursors and their subsequent appearance in the circulation at 2 days after ^{59}Fe injection was actually enhanced in irradiated C3H mice, and this discounts reduced red cell production as being a principal cause of anemia. Furthermore, erythropoietin production is not exclusively confined to renal tissue and may be generated in greater amounts during hemolysis from extrarenal sources such as liver Kupffer cells and resident bone marrow macrophages (Peschle *et al.* 1978, Bondurant and Korny 1986, Rich 1987).

The maintenance of adequate hemopoiesis throughout the post-transplant period is of primary concern in BMT recipients. A long-term reduction in bone marrow stem cell renewal related to dose of transplanted marrow cells has recently

been demonstrated in TBI-treated C3H mice (Mauch and Hellman 1989). In the present study we found normal peripheral white blood cell counts but, in spite of an apparent increase in erythroid activity, the persistent anemia does signify an insufficient compensation to replace lost cells. Here the bone marrow and spleen represent secondary vulnerable sites where enhanced erythropoiesis to anemic stress may be impaired by late radiation injury. Our findings of reduced spleen weights after TBI (about 50 per cent of controls) signifies the extra demand placed on erythropoietic tissues that may already be compromised by previous irradiation. Future studies aimed at comparing the effects of localized bilateral kidney irradiation and TBI would help to clarify the role of renal and extrarenal tissue damage in contributing to anemia.

Of additional interest is the comparison between the pathology of radiation nephropathy as described here and the type of renal damage produced by other known nephrotoxic agents. *Cis*-platinum, for example, appears to exert most damage to the tubule epithelium, presumably due to the preferential concentration and metabolism of toxins in these cells (Abelson and Garnick 1982, Humes and Weinberg 1986). While hemolytic anemia is not a typical accompaniment to tubular injury in such cases, enhanced renal complications as seen experimentally in mice (Stewart *et al.* 1986, 1987) and rats (Jongejan *et al.* 1987, Moulder *et al.* 1987b) remains possible when *cis*-platinum is applied in combination with clinical TBI (Tarbell *et al.* 1988). In contrast to *cis*-platinum, mitomycin-C has a nephrotoxic effect similar to radiation; glomeruli rather than tubules become the most sensitive to injury and HUS becomes the clinical complication (Verweij *et al.* 1987). Hence glomerular damage, endothelial in particular, again represents the major culprit responsible for renal insufficiency and microangiopathic hemolysis. Such comparisons help provide a mechanistic approach to understanding the way in which certain chemotherapeutic drugs interact with radiation to affect the final development and magnitude of kidney injury following combined cancer therapy.

In summary, a strong dependency on radiation dose and genetic strain was found for the timing of late lethality in mice after split-dose TBI and bone marrow rescue. Pleural effusions represented a life-threatening complication but only at a high total dose of 15 Gy in $B6AF_1$ mice. Nephrotoxicity, as shown by glomerular lesions, uremia and an associated hemolytic anemia, was the most serious and prevalent problem at later times and at lower doses (11–13 Gy). This constitutes the first detailed description of a radiation-induced HUS in rodents and offers a useful laboratory model for addressing the corresponding problem in BMT patients.

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