Radiation Nephropathy

By Eric P. Cohen and Mike E. C. Robbins

The pronounced radiosensitivity of renal tissue limits the total radiotherapeutic dose that can be applied safely to treatment volumes that include the kidneys. The incidence of clinical radiation nephropathy has increased with the use of total-body irradiation (TBI) in preparation for bone marrow transplantation and as a consequence of radionuclide therapies. The clinical presentation is azotemia, hypertension, and, disproportionately, severe anemia seen several months to years after irradiation that, if untreated, leads to renal failure. Structural features include mesangiolysis, sclerosis, tubular atrophy, and tubulointerstitial scarring. Similar changes are seen in a variety of experimental animal models. The classic view of radiation nephropathy being inevitable, progressive, and untreatable because of DNA damage-mediated cell loss at division has been replaced by a new paradigm in which radiation-induced injury involves not only direct cell kill but also involves complex and dynamic interactions between glomerular, tubular, and interstitial cells. These serve both as autocrine and as paracrine, if not endocrine, targets of biologic mediators that mediate nephron injury and repair. The renin angiotensin system (RAS) clearly is involved; multiple experimental studies have shown that antagonism of the RAS is beneficial, even when not initiated until weeks after irradiation. Recent findings suggest a similar benefit in clinical radiation nephropathy.

SUFFICIENT EXPOSURE of kidneys to ionizing radiation will cause loss of function and may lead to renal failure. The first recorded knowledge of this effect was in 1906, barely a decade after the discovery of x-rays in 1895.[1] As opposed to therapeutic irradiation; diagnostic radiation does not pose this risk. By way of example, a typical abdominal computed tomography scan delivers about 20 mGy to the irradiated organs. This is more than 1,000-fold lower than the irradiation dose needed to cause radiation nephropathy.

CLINICAL RADIATION NEPHROPATHY

Clinical Occurrence

Radiation nephropathy was well documented in a large case series published by Kunkler et al[2] over 50 years ago. These were men who had undergone therapeutic irradiation for seminomas. Radiation nephropathy occurred in about 20% of sufficiently irradiated subjects, and could take various clinical forms (Fig 1). The most common was acute radiation nephritis, which presented clinically as azotemia, hypertension, and anemia starting at 6 to 12 months after irradiation. *Nephritis* appears to be a misnomer in that the histologic features are not inflammatory. Numerous cases were reported over the next 20 years. Over this time, radiation nephropathy became less and less of a clinical problem, not only because the threshold radiation dose for renal injury became defined, but also because of the advent of better chemotherapy. This is particularly evident for the case of advanced seminomas, which are radiosensitive cancers, yet for which chemotherapy is now a better option than is radiation therapy.[3]

Another cause of classic radiation nephropathy is irradiation of kidney transplants. That therapy was used formerly as treatment for acute kidney transplant rejection, but now it is not used for that purpose because it has been shown to be ineffective.[4]

The use of total body irradiation (TBI) in the preparation for bone marrow transplantation (BMT) has led to a resurgence of radiation nephropathy.[5] Clinically, the features of this variant are similar to those of acute radiation nephritis as published by Luxton[6] (Fig 1). The more severe variants have features of thrombotic microangiopathy, or hemolytic uremic syndrome/thrombotic thrombocytopenic purpura. In the first report of this complication of BMT in 1978, the likely role of irradiation was already suggested but it was not until 10 years later in 1988 that the role of irradiation was acknowledged.[7] There are now over 100 cases published on this form of radiation nephropathy, which we have called *BMT nephropathy*.

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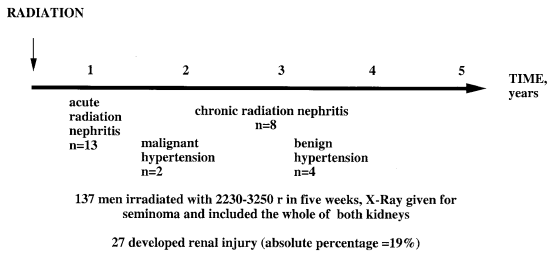

Fig 1. A schema of the occurrence of radiation nephropathy according to time after irradiation, as defined by Luxton and adapted from his data. A total of 137 men were irradiated with 2,230 to 3,250 rads in 5 weeks. X-rays were given as treatment for seminomas and included the whole of both kidneys: 27 developed renal injury (absolute percentage 20%). Reprinted with permission.[110]

The use of radioisotope therapies is an additional mechanism for clinical radiation nephropathy. In Europe, radioactive yttrium tagged to a somatostatin receptor ligand has been used to treat neuroendocrine malignancies. The isotope-protein conjugate is filtered at the glomeruli, and then reabsorbed by tubular epithelium. There, the β emission of the isotope is concentrated, and a number of cases of radiation nephropathy related to this therapy have been described.[8] The occurrence of this complication appears to depend on interplay of the pharmacokinetics of the conjugate and radioactive decay of the isotope. Specifically, the conjugate needs to be of the right size for its filtration, and the isotope needs to decay slowly enough for its effect to be evident. Glomerular filtration may not be required for all radioisotope nephropathies, and radiation nephropathy also has appeared as a complication of radioimmunotherapy by rhenium conjugated to an immunoglobulin that is specific for the CD-66 epitope of lymphocytes.[9]

In radiation nephropathy, as in normal tissue radiation injury in general, it is not possible to predict which subjects will develop the complication. As noted, only 20% of the patients in Luxton's[6] case series developed radiation nephropathy. Why these men were susceptible—and the other 80% were not—remains unexplained. There are rare syndromes of radiation sensitivity such as ataxia telangiectasia, but these are not clinically frequent. The role of the angiotensin converting enzyme (ACE) gene polymorphisms was investigated and found to be unhelpful in predicting renal failure after BMT.[10]

It is possible that radiation nephropathy could occur after a nuclear accident or because of nuclear terrorism. Exposures that would cause this would have to be in the 5 to 10 Gy range. Doses less than 5 Gy would not materially affect the kidneys, whereas doses greater than 10 Gy would cause rapid gastrointestinal death.

Threshold Dose

The accepted threshold dose of photon irradiation that will cause radiation nephropathy is exposure of both kidneys to a total dose of 23 Gy, fractionated in 20 doses over 4 weeks.[11] If only one kidney is irradiated with a threshold or higher dose, radiation injury will occur in that kidney, but kidney failure from radiation nephropathy per se will not occur. However, the unirradiated kidney is likely to become damaged from the renin-mediated hypertension that occurs because of the severe unilateral renal scarring.[12]

In the case of radiation nephropathy after BMT, a 10 Gy TBI single dose of x-rays will cause this form of radiation nephropathy, as will 14 Gy fractionated over 3 days.[6]

As for the radioisotope-induced radiation nephropathy, the exact delivered doses are not always well defined. In the case of the rhenium conjugate used for radioimmunotherapy, the total kidney dose from the radionuclide is estimated at 7 Gy.[9] This dose would not in itself be sufficient for kidney injury, but because it was added to 12 Gy TBI in the patients of that report, it provides an additional nephrotoxic effect.

Clinical Features and Management

The clinical presentation is azotemia, hypertension, and, disproportionately, severe anemia in a subject who has received a sufficient dose of therapeutic irradiation. In the case of acute radiation nephropathy and its congeners, this is at 4 months or more after irradiation. Untreated, there is evolution to renal failure, and survival on chronic dialysis is poor.[13] Kidney biopsy examination shows mesangiolysis, as well as atrophy and tubulointerstitial scarring (Fig 2). Severe cases have features of thrombotic microangiopathy. In those cases, plasmapheresis has been used but appears to have no benefit on the renal manifestations.[14]

Management of radiation nephropathy includes attention to control of blood pressure and the use of ACE inhibitors or angiotensin (AII) receptor

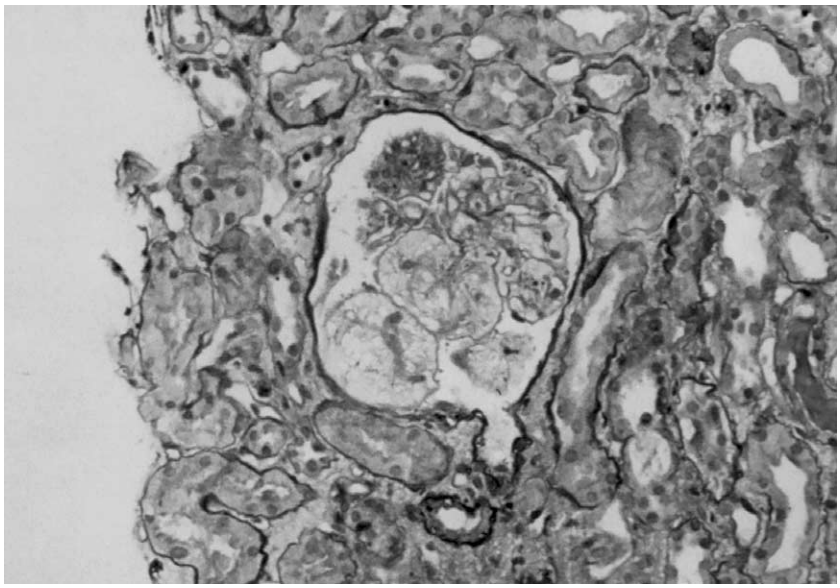

Fig 2. Light microscopy of a kidney biopsy specimen in a case of BMT nephropathy. There is mesangial cell loss (mesangiolysis) and there is interstitial expansion and tubular separation.

blockers. The same principles apply here as they do to any chronic kidney disease. There are no controlled studies of the use of ACE inhibitors or AII blockers, although we advise their use in such cases. In addition, one case report shows clear-cut arrest of progressive loss of kidney function in a case of radiation nephropathy treated with an AII blocker.[15] The anemia of radiation nephropathy can be treated with epoetin.

RADIATION TOLERANCE OF THE KIDNEY

The kidney is a radiosensitive organ. Clinically, the tolerance dose (for 5% complications in 5 years) for the kidney is approximately 20 Gy, as compared with values of 50 and 60 Gy for the bladder and brain, respectively[16,17]; doses of approximately 25 to 30 Gy given in conventional 2-Gy fractions to the total renal mass are likely to lead to chronic renal failure.[18] Experimental studies indicate that the kidney has an extensive capacity for repair of sublethal radiation damage. The size of the dose per fraction markedly influences the total tolerance dose[19,20]; there is a marked increase in the tolerance dose with decreasing size of the dose per fraction. Kidney fractionation data can be analyzed using the linear quadratic formula[21]:

$$E = n(\alpha d + \beta d^2)$$

where the effect (E) is a linear and quadratic function of the dose per fraction (d) and a function of the fraction number (n). This equation allows determination of the α/β ratio, a measure of the bendiness of the underlying putative target cell survival curve. In general, experimental kidney fractionation data have been well described by the linear quadratic model, with most studies indicating an α/β ratio of 2 to 3 Gy,[19,20,22] a value similar to that observed in other late responding tissues including the spinal cord and lung.[23] At doses of less than 1 to 2 Gy per fraction, the tolerance dose appeared to be less than predicted using the linear quadratic model.[20] This may reflect incomplete DNA repair between fractions because short interfraction intervals of 5 hours were used. However, a reduction in repair capacity after multiple dose fractions owing to diminished induction of molecular repair mechanisms or the presence of a small population of radiosensitive cells cannot be excluded.[24]

Retreatment and Residual Injury

The absence of any measurable renal dysfunction at the time of re-treatment cannot be interpreted as a lack of latent or residual injury. Experimental studies show that doses of radiation too low to produce overt renal damage do significantly reduce the tolerance to re-treatment.[25] These data are consistent with there being permanent genetic injury caused by the initial treatment that is simply compounded by the re-treatment and imply that

Table 1. The Morphologic Features Observed in the Various Components of the Kidney After Irradiation in Different Species

	Species					
	Mouse	Rat	Dog	Pig	Monkey	Human
Glomerular features						
Nuclear enlargement	+ (>3 mo)	–	–	+ (2 wk)	–	–
Inflammation	–	–	–	+ (2 wk)	–	–
Mesangial proliferation	–	–	–	+ (6 wk)	+ (6 mo)	–
Mesangiolysis/thrombosis	+ (10 mo)	+ (2–3 mo)	+ (2 mo)	+ (3 mo)	+ (6 mo)	+
Glomerulosclerosis	+ (10 mo)	+ (2–3 mo)	+ (3 mo)	+ (5 mo)	+ (6 mo)	+
Vascular features						
Thrombosis	+ (3 mo)	–	–	+ (4 mo)	+	
Medial hyperplasia	–	–	+ (2 mo)	+ (3 mo)	+ (6 mo)	+
Intimal thickening	–	–	–	–	+ (6 mo)	+
Tubular/interstitial features						
Reactive changes	+ (6 mo)	+ (6 wk)	+ (2 wk)	+ (6 wk)	+ (4 mo)	+
Tubulolysis (6 mo)	+ (6 wk)	+ (4 wk)	–	–	–	+
Tubular atrophy/interstitial fibrosis	+ (6 mo)	+ (2–3 mo)	+ (2 mo)	+ (3 mo)	+ (4 mo)	+

NOTE. Data in parentheses refer to the time after irradiation at which these changes have been observed.

re-treatment of the kidneys should be viewed with extreme caution.

MORPHOLOGIC CHANGES

The progressive reduction in renal function observed after kidney irradiation is associated with concomitant time- and dose-dependent alterations in all components of the nephron leading to glomerulosclerosis and/or tubulointerstitial fibrosis.[26] Serial determinations of morphologic changes indicate that primarily glomerular but also tubular alterations occur in the early stages of radiation nephropathy. Irradiated glomeruli show lesions that appear to develop regardless of the examined species (Table 1). These include variable capillary loop thickening with subendothelial expansion and basement membrane duplication (Fig 3). The mesangium shows lysis of cells and matrix in continuity with the subendothelial space; capillary loop collapse and mesangial sclerosis eventually de-

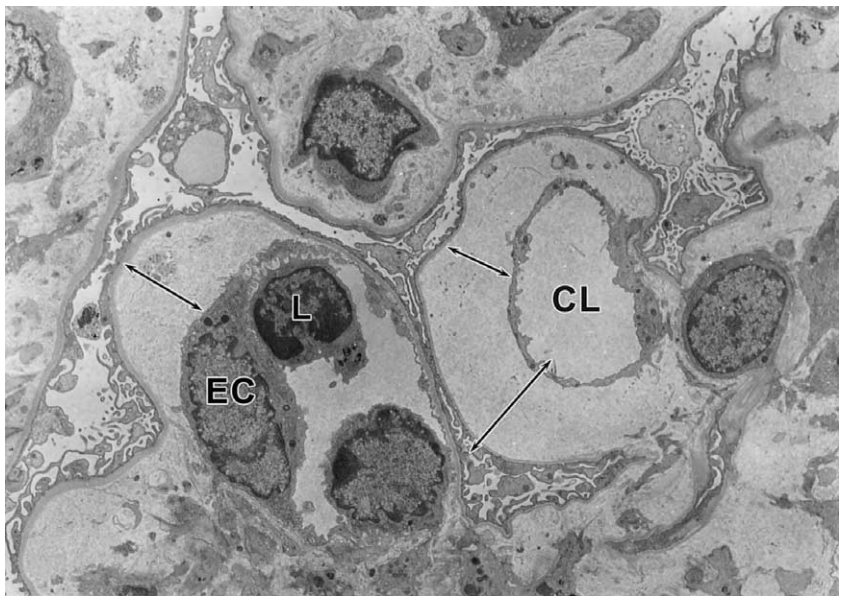

Fig 3. Glomerular capillary loops showing widened subendothelial space (double-ended arrows); capillary tuft at left is lined by activated endothelial cells (EC) with close leukocyte (L) attachment. Capillary lumen (CL) 9 weeks after irradiation of pig kidneys with a single dose of 9.8 Gy γ rays (×3,750). This ultrastructural appearance is very similar to that of human BMT nephropathy. Reprinted with permission.[111]

velop. Mice and pigs show prominent nuclear enlargement in a subset of glomerular, likely endothelial, cells.[27,28] Adherence of mononuclear cells, presumably lymphoid in nature, to glomerular capillary loops consistently occurs in pigs.[28] Dose-dependent increases in ectatic glomerular capillaries, capillary adhesions, and thrombi have been reported in monkeys and pigs.[29,30]

There are pronounced mesangial changes. Diffuse and severe mesangial hypercellularity is observed in monkeys but appears more focal in pigs. There is little evidence of mesangial hypercellularity in mice and rats.[26] However, other studies have noted increased mesangial cell proliferation.[31] All species exhibit mesangial sclerosis.

Tubular interstitial injury is an additional feature of radiation nephropathy. At 6 weeks after irradiation of pig kidneys, small cortical foci of reactive-appearing epithelium are seen, characterized by nuclear enlargement, nuclear predominance, and basophilic cytoplasm.[26] As injury progresses, tubular atrophy develops, characterized by small tubular cells enveloped by a thick, irregular basement membrane. A distinctive subcapsular accentuation of these tubular lesions is prominent in pigs[32] and also has been reported in humans.[33] An additional type of tubular injury termed *tubulolysis* has been reported[26] in which there is apparent lysis of tubular cells leaving an empty or denuded profile of tubular basement membrane. Tubulolysis has not been observed in monkeys, nor has it been described in humans. In contrast, interstitial fibrosis with mild interstitial inflammation is a common feature of advanced disease in all species.

Studies in the pig have revealed a striking and progressive pattern of fibrosis with narrowing of the glomerulotubular neck after irradiation[34] (Fig 4). Irradiated kidneys showed distinctly narrowed tubular necks, apparently formed by interstitial collagen with compression of the tubular epithelium and narrowing of the tubular neck lumen. These narrowed necks increased in prevalence with time; 20 weeks after irradiation, the average neck diameter reduction was 60% as compared with nonirradiated controls, a percentage consistent with a reduction in flow and pressure at this critical point of the nephron. These stenotic necks also have been observed in the irradiated rat kidney, and their development is blunted significantly by the therapeutic use of captopril.[35] Glomerulotubular neck narrowing thus directly may reduce the glomerular filtration rate (GFR) of an individual nephron, providing an anatomic and functional link between tubulointerstitial fibrosis and loss of glomerular function.

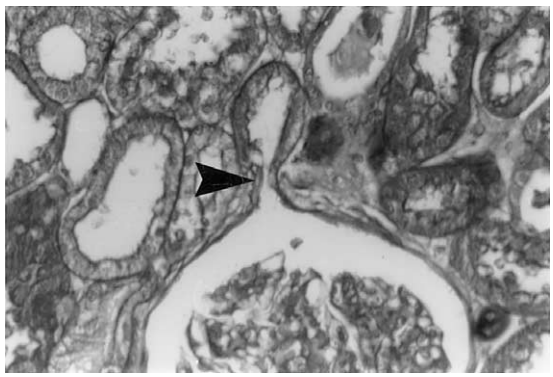

Fig 4. Light micrograph of a glomerulotubular neck stenosis in experimental radiation nephropathy (×400). The narrowed neck, indicated by an arrowhead, was confirmed by serial sections. Stenotic necks have been seen in radiation nephropathy and in other renal diseases, including immunoglobulin A nephropathy. Reproduced with permission.[34]

Scanning electron microscopy has shown tuft-to-capsule adhesions in experimental radiation nephropathy,[36] and it is possible that these contribute to periglomerular fibrosis and formation of stenotic glomerulotubular necks. These stenotic necks may be an intermediate step in the formation of atubular glomeruli. The occurrence of atubular glomeruli in radiation nephropathy has been documented by scanning electron microscopy.[37] In this model as well as others, atubular glomeruli may explain the lack of good correlation between kidney function and traditional criteria for glomerular injury—an apparently intact glomerulus may no longer be a filtering one.

A variety of vascular lesions develop in experimental models of radiation nephropathy. Arteriolar and arterial thromboses develop in parallel with glomerular thrombosis in rats and monkeys, particularly at high doses. Thrombotic lesions are rare in mice and pigs, even after high radiation doses.[38,39] Occlusive but nonthrombotic vascular lesions develop in pigs, monkeys, rabbits, and dogs. Pigs, rabbits, and dogs develop arteriolar and arterial medial hyperplasia. In contrast, monkeys develop occlusive intimal thickening without medial alterations. These types of chronic occlusive lesions do not appear to develop in the mouse and rat kidney after renal irradiation. Thus, mere vas-

cular injury cannot be the sole mechanism for radiation nephropathy.

The relative contribution that these various lesions make to the development of radiation nephropathy remains ill defined. Because radiation-induced glomerular changes appear diffuse and are seen before tubular alterations[29,32,40] they have been proposed to be of primary importance in the development and progression of radiation nephropathy. However, chronic renal failure is observed primarily in those animals in which glomerular injury is combined with severe tubular injury and tubulointerstitial fibrosis.[41] In clinical chronic renal disease the degree of tubulointerstitial fibrosis is correlated closely with the degree of renal dysfunction[42]; no such correlation is seen with glomerular changes.[43] Indeed, radiation nephropathy, evidenced in terms of dose-dependent reductions in renal function and tubulointerstitial fibrosis, has been observed in the absence of any significant glomerular lesions.[44]

WHOLE-ORGAN RADIATION NEPHROPATHY

Functional Changes

There is a wealth of experimental data on functional changes in radiation nephropathy.[26] Studies in the monkey,[41] pig,[45,46] and rat[47] have reported pronounced changes in GFR and effective renal plasma flow within several weeks of irradiation. There is evidence for an initial hyperemic response in GFR and effective renal plasma flow followed by a dose-dependent decline in GFR and effective renal plasma flow within 6 to 8 weeks of irradiation.[45] Measurements of renal function assessed as blood urea nitrogen show a dose-dependent decline in renal function that is predominantly progressive in nature and if untreated leads to renal failure.[48]

Studies in the pig suggest that the overall time sequence of changes in renal hemodynamics observed after unilateral[45,49] and bilateral irradiation,[46] or irradiation of a single hypertrophied kidney,[50] is essentially the same. A similar pattern of response also has been seen in the monkey after kidney irradiation.[41] Although there is some evidence that mice are relatively resistant to radiation-induced renal injury and exhibit radiation nephropathy much later than rats, interpretation of these data is complicated by likely strain-dependent differences in renal response to radiation. Thus, although C57BL mice failed to show an increase in blood urea nitrogen until approximately 1 year after TBI,[51] CBA mice exhibited dose-dependent decreases in renal function within 12 weeks of irradiation.[52] Overall, these findings imply a common renal response to radiation and thus it is likely that common pathogenic mechanisms are involved.

The earliest physiologic change of the irradiated kidney appears to be an increase in the glomerular permeability to albumin, P_{alb}, evident within 1 hour of 9.5-Gy single-fraction TBI to rats.[51] This response is measured by an in vitro assay of glomerular albumin permeability. Enhanced glomerular albumin permeability is seen within 5 minutes of in vitro irradiation of isolated glomeruli, which appears to rule out a systemic nonrenal origin for this permeability change. This permeability effect may be dependent on cyclic adenosine monophosphate signaling pathways.[53] Because it is such an early response, this glomerular permeability response does not fit with the classic paradigm for radiation injury, which typically is held to depend on DNA damage leading to cell death.

It is noteworthy that this change in glomerular permeability diminishes with time after single-fraction 9.5-Gy TBI. That is, in glomeruli harvested from irradiated rats at 40 days after irradiation, the change in P_{alb} is no longer apparent. It is at that time that urinary protein increases in irradiated rats, which suggests that proteinuria in radiation nephropathy is not simply caused by enhanced glomerular protein leak. It is possible that reduction of endocytic resorbtion of filtered protein could play a role in proteinuria. Irradiated kidney brush border membranes have substantial reductions in albumin binding, and this could be related to defects of the megalin-cubulin protein complex of the brush border.[54] Deficient function of that complex could lead to albuminuria.

Additional radiation-induced changes in function include anemia, the severity of which is disproportionate to the degree of azotemia. This radiation-induced anemia is characterized as either a severe normochromic normocytic anemia with a low absolute reticulocyte count suggesting inhibition of erythropoietin production,[46] or a microangiopathic hemolytic anemia.[55] These differences in the type of radiation-induced anemia likely reflect different target sites. The critical site for erythropoietin production is the interstitial peritubular fibroblast,[56] whereas the hemolytic-type anemia likely reflects damage to the glomerular capillary

tufts; these show marked disruption after irradiation.[57] These 2 causes for anemia may explain the disproportionate severity of anemia in radiation nephropathy.

Hypertension frequently is associated with radiation nephropathy,[58] however, the mechanisms responsible remain poorly defined. Possible pathogenic mechanisms include a reduction in blood flow with resultant ischemia, or a renoprival lesion in which irradiation causes direct tubular damage. Hypertension in radiation nephropathy also has been attributed to the reduction in renal circulation produced by damage to and thickening of the intrarenal arteries and glomerular arteriolar walls.[59] However, hypertension can develop in the absence of these vascular lesions.[60] Hypertension from a renoprival mechanism could result from tubulointerstitial injury and fibrosis, which could cause impaired natriuresis and subsequent volume expansion.[61] The renin-angiotensin system (RAS) has been implicated in radiation-induced hypertension[62]; radiation-induced vascular damage might lead to ischemia, activation of the RAS, and increased production of AII. However, studies have failed to identify a radiation-induced activation of the RAS.[63] Studies in the rat BMT model of radiation nephropathy suggest a role for nitric oxide; irradiation led to a progressive reduction in nitric oxide production, assessed as urinary cyclic guanosine monophosphate.[64]

CELLULAR RADIATION INJURY

Renal tubular epithelial cells clearly are radiosensitive; a D_0 (radiation dose that reduces the surviving fraction to 37% of initial value) of 1.5 Gy, similar to that found for other mammalian cells, has been reported.[65] Similar values also have been noted for endothelial cells irradiated in vitro.[66] Moreover, radiation-induced increases in glomerular and tubular cell proliferation, likely in response to radiation-induced cell kill, have been noted within several weeks of irradiation.[67-69] The specific type of cells involved in this proliferative response include glomerular capillary endothelial cells, mesangial cells, and tubule epithelial cells.[28,68,70] This proliferation is not associated with increased cell number, indicating that cell proliferation is matched by cell loss.[69,71]

Radiation-induced apoptosis has been observed in rat mesangial cells irradiated in vitro[72] and in neonatal rat kidneys irradiated in vivo[73] after a single dose of 5 Gy. However, the pathogenic role of apoptosis, and its relative importance compared with necrosis, in radiation nephropathy is unclear.

Radiation-induced DNA damage leading to cell death is only one facet of overall cellular and tissue response. Ionizing radiation, in common with other forms of stress, causes pronounced changes in cell phenotype via activation of signal transduction pathways and transcription factors[74] that lead to alterations in cell phenotype. Pronounced changes in kidney cell phenotype have been observed after in vitro irradiation.

RADIATION-INDUCED CHANGES IN KIDNEY CELL PHENOTYPE

Mesangial Cell

Irradiating rat mesangial cells with single doses of 5 to 20 Gy γ rays led to isoform-specific alterations in gene expression of the profibrogenic cytokine transforming growth factor β (TGF-β). *TGF-β_1* messenger RNA (mRNA) levels showed a dose-independent increase 24 to 48 hours postirradiation. In contrast *TGF-β_3* mRNA levels showed a progressive dose-independent decrease over the same time period.[75] These changes were associated with a concomitant increase in gene expression for several extracellular matrix components, including fibronectin and biglycan. However, no alterations were noted in collagen I, collagen III, or decorin mRNA.[75] Subsequent studies revealed modest increases in TGF-β protein secreted by mesangial cells 24 hours postirradiation; this increase was primarily in latent, not active, TGF-β protein.[76] Radiation-induced changes in the expression of gene products associated with the regulation of extracellular matrix turnover also have been reported. Irradiating rat mesangial cells with single doses of 0.5 to 20 Gy γ rays resulted in time- and dose-dependent increases in fibronectin and plasminogen activator inhibitor-1 (PAI-1) immunoreactive protein, and differential changes in matrix metalloproteinase (MMP) expression; active MMP-2 levels increased whereas MMP-9 levels were unaltered.[77] Additional data showed increased secretion of tissue inhibitor of MMP-2 without a concomitant increase in secretion of plasminogen activators.[77]

Tubule Epithelial Cell

In vitro irradiation of rat kidney tubule epithelial cells also leads to marked changes in the expres-

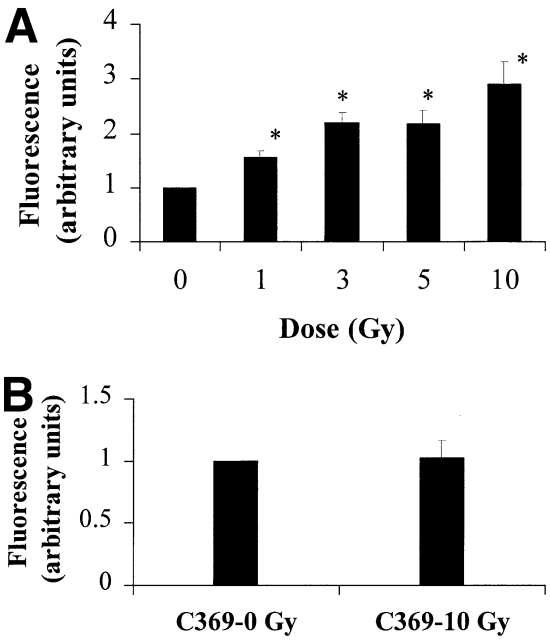

Fig 5. Radiation stimulates ROS generation in rat tubule epithelial cells. Cells were preloaded for 30 minutes with the oxidation-sensitive fluorescence probe 2′7′-dichlorodihydofluorescein diacetate (H_2DCFDA), washed, irradiated with 0 to 10 Gy γ rays, and the relative fluorescence was measured at 1 hour postirradiation. The oxidation-insensitive fluorescence probe C369 was used as control. The top panel shows data generated using H_2DCFDA; the lower panel shows data generated using C369. Mean ± SE; $n = 3$ (3 independent experiments); *$P < .05$. Reprinted with permission.[79] © 2001 American Association for Cancer Research.

sion of gene products involved in both matrix degradation and synthesis. Thus, irradiating NRK52E cells resulted in significant increases in *collagen I*, *PAI-1*, and *TGFβ_1* gene expression. Further, active MMP-2 levels increased whereas MMP-9 levels were unaltered.[78] More detailed mechanistic studies suggest that these radiation-induced changes in kidney cell phenotype are caused, in part, by increased oxidative stress.[79] Irradiating NRK52E cells led to dose-dependent increases in intracellular reactive oxygen species (ROS) generation 1 hour postirradiation (Fig 5). Dose-dependent increases in PAI-1 immunoreactive protein were observed 48 hours after irradiation. This increase in PAI-1 expression was abolished by increasing intracellular soluble thiol pools after incubation with *N*-acetylcysteine. In addition, overexpression of catalase using an adenovirus-based gene transfer approach inhibited radiation-induced increases in PAI-1 expression (Fig 6), suggesting a mechanistic role for hydrogen peroxide in regulating PAI-1 expression after oxidative insult.

IN VIVO MEDIATORS AND MECHANISMS

In recent years the characterization of radiation-induced normal tissue injury has undergone a paradigmatic shift. Pathophysiologic data from a variety of late responding tissues indicate that the expression of radiation-induced normal tissue injury involves complex and dynamic interactions between several cell types within a particular organ.[28,80-82] These now are viewed not as passive bystanders, merely dying as they attempt to divide, but as active participants in an orchestrated, yet limited, response to injury. Thus, there appear to be mediators of this normal tissue radiation response and these mediators may provide targets for suc-

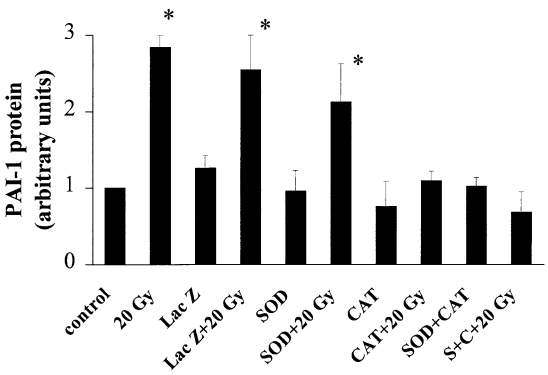

Fig 6. Overexpression of catalase (CAT) but not manganese superoxide dismutase (MnSOD) leads to inhibition of the radiation-induced increases in PAI-1 immunoreactive protein levels in rat tubule epithelial cells. Cells were transduced with either Ad*LacZ* (100 MOI), Ad*MnSOD*, and/or Ad*CAT* (30 MOI). Twenty-four hours later these cells and nontransduced cells were placed in serum-free medium for 24 hours before irradiation with a single dose of 20 Gy γ rays; control cells received sham irradiation. Conditioned medium was collected at 48 hours postirradiation. Equal amounts of medium volume (based on total DNA amount) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The protein level of PAI-1 was analyzed by Western immunoblotting using polyclonal rabbit anti–PAI-1 antibodies. Densitometric quantification shows the effect of MnSOD and/or CAT gene transfection on PAI-1 protein induced by radiation in tubule epithelial cells. Mean ± SE; $n = 3$; *$P < .05$ as compared with the protein level of PAI-1 observed in control cells. Reprinted with permission.[79] © 2001 American Association for Cancer Research.

cessful therapies. Clearly, the development and progression of radiation nephropathy involves multiple cell types, including the glomerular capillary endothelial cell, the mesangial cell, and the tubule epithelial cell.

Glomerular capillary endothelial cell damage represents an important event in radiation nephropathy. Attachment of neutrophils to the injured endothelium is an early feature.[28] Putative mechanisms include a radiation-induced release of chemotactic factors from the endothelial cells[83] and a radiation-induced increase in the synthesis and/or up-regulation of cell adhesion molecules such as E-selectin[84] and intercellular adhesion molecule 1.[85] The latter has been shown to play an important role in radiation-induced lung injury; intercellular adhesion molecule $1^{-/-}$ mice showed both reduced inflammatory cell infiltration and pulmonary fibrosis after lung irradiation.[86] The vascular and glomerular thromboses described in radiation nephropathy could well derive from radiation-induced cellular changes that favor coagulation, such as decreased PGI_2 production and increased release of vWf.[87] However, the in vivo radiation-induced changes in glomerular endothelial cell vWf expression were not sensitive to changes in total dose or dose per fraction, and thus they cannot be linked quantitatively to the development of radiation nephropathy.[88] Oikawa et al[89] provided additional evidence for the role of the fibrin-fibrinolytic system by showing increased glomerular *PAI-1* mRNA in a rat model of radiation nephropathy, and the attenuation of that increase by either ACE inhibitors or an AII receptor blocker. Increased PAI-1 may not only prevent fibrinolysis, but also could promote fibrosis via inhibition of plasmin-mediated matrix degradation.

Cellular mechanisms of fibrosis also involve activation of fibroblasts into myofibroblasts, contractile fibroblasts that express α-smooth muscle actin (α-SMA) and that represent the main source of increased extracellular matrix deposition seen in renal fibrosis and in other forms of tissue fibrosis.[90,91] Glomerular mesangial cells acquire α-SMA staining when there is inflammatory injury or proliferation.[92] Distinct mesangial α-SMA expression was observed within 2 weeks of irradiation of the pig kidney, with peak expression seen at 4 weeks,[93] a time period during which radiation-induced increases in glomerular cell proliferation have been reported.[68] Of interest, glomerular $TGF\beta_1$ expression was a late and unimpressive phenomenon in this model, suggesting that TGF-β_1 might not contribute significantly to glomerular scarring, at least in this model. Increased latent but not active $TGF\beta_1$ production was observed in glomerular lysates 50 to 63 days after bilateral irradiation of the rat kidney, a timeframe consistent with $TGF\beta_1$ having a contributory rather than causal role in radiation nephropathy.[94] Of interest, administration of an AII receptor blocker completely inhibited this increase in glomerular $TGF\beta_1$ production, indicating a role for AII in mediating the induction in $TGF\beta_1$.

Glomerular fibrin deposition was reported in the irradiated pig kidney, occurring within 4 weeks of irradiation and remaining elevated throughout the development of radiation nephropathy.[93] This was associated with significant tubular fibrin deposition, probably derived from the injured glomerular tufts via downstream egress. Tubular fibrin and its smaller catabolite fibrin peptides (molecular weight $\sim$ 1,500 da) could contribute to tubulointerstitial fibrosis by crossing the tubular basement membrane of denuded segments with resultant stimulation of interstitial fibroblasts.[95] In these studies, $TGF\beta_1$ expression was predominantly in the tubular epithelial cytoplasm with interstitial expansion and the appearance of interstitial cells expressing $TGF\beta_1$. This sequence is consistent with a role for $TGF\beta_1$ in the later phases of radiation nephropathy, but not as an early mediator.

Additional information regarding specific cell types and potential mediators involved in radiation-induced tubulointerstitial fibrosis has come from studies in the rat. After irradiation of a single hypertrophied kidney, Robbins et al[44] observed time- and dose-dependent increases in tubular cell atrophy and lysis. These changes were associated with increased interstitial staining for α-SMA, collagen III, and fibronectin. There was a significant increase in tubular $TGF\beta$ staining, confirming previous observations in the pig. However, this was observed only at 8 weeks postirradiation. In contrast, the degree of interstitial $TGF\beta$ increased progressively after irradiation. Radiation-induced activation of interstitial myofibroblasts with resultant collagen deposition also was reported after unilateral irradiation of the rat kidney.[96]

An increasing body of evidence supports a contributory role of increased ROS generation and oxidative damage in the pathogenesis of chronic

renal disease.[97] ROS also may be of pathogenic importance in radiation nephropathy. The biologic effects of ionizing radiation result from energy deposition in irradiated cells and subsequent acute generation of short-lived ROS.[98] However, treatment of Chinese hamster ovary cells with 10 Gy has been shown to lead to persistent oxidative stress.[99] In view of the difficulty in measuring ROS in vivo, Robbins et al[100] adopted an indirect immunohistochemical approach using a monoclonal antibody specific for 8-hydroxy-2′-deoxyguanoisine, one of the most commonly used markers for evaluation of oxidative DNA damage. Sham-irradiated kidneys showed little evidence of DNA oxidation over the experimental period. In contrast, kidney irradiation led to a marked dose-independent increase in glomerular and tubular cell nuclear DNA oxidation. This increase was evident at the first time point studied (ie, 4 weeks after irradiation), and persisted for up to 24 weeks postirradiation. DNA oxidation in the irradiated kidney only was seen in apparently viable glomerular and tubular cells.[100] These data support the hypothesis that renal irradiation is associated with a chronic and persistent oxidative stress. This might serve, in part, as a mechanism for the chronic modulation of kidney cell phenotype seen after irradiation; several putative mediators such as PAI-1, MMP-2, and TGFβ are redox-regulated.[79,101,102]

RAS

The role of the RAS in radiation nephropathy is supported by multiple studies that show that antagonism of the RAS is beneficial, and also by evidence that AII excess exacerbates radiation nephropathy. Captopril was used successfully to treat established experimental radiation nephropathy, and this benefit is shared by the AII blocker L-158,809.[48,103] Prevention studies, in which drug is started at or just before irradiation, confirm the benefits of RAS antagonism, by ACE inhibitors or AII blockers.[58,89,104] It is worth noting that captopril does not act as a classic radioprotector because its start can be delayed until 3 weeks after irradiation, without significant loss of its long-term preventive benefit.[105]

It might thus be expected that the RAS would in some way be activated in radiation nephropathy. Thus far, evidence for this is meager. Although Fisher and Hellstrom[60] found enhanced juxtaglomerular indices at 15 weeks after 11 Gy local kidney irradiation in rats, Robbins et al[46] found no increase in blood renin in pigs subjected to 8 to 12 Gy local kidney irradiation. In the rat model of radiation nephropathy established by Moulder, normal to low plasma renin levels were found, and no change in plasma or intrarenal AII levels were found.[63] We recently have found an apparent increase in AII receptor abundance and affinity in irradiated rat kidneys at 6 weeks after 17 Gy TBI, but these data are preliminary and require confirmation.

In nephrology, the concept of a generally not activated RAS has been described as a paradox of chronic kidney disease.[106] That is, it is paradoxic that activation of the RAS is not apparent in chronic kidney disease, yet antagonism of the RAS is beneficial. In a model of renal disease related to nitric oxide depletion, Verhagen et al[107] have observed normal activity of the RAS that coincides with a benefit of AII antagonism. Conceivably, in radiation nephropathy as in any chronic renal disease, experimental or clinical, normal activity of the RAS is a bad thing, and its inhibition is thus desirable.

The mechanism of the benefit of ACE inhibitors or AII blockers in radiation nephropathy is not established. Mere control of the blood pressure, or control of proteinuria, is not sufficient because an 8-fold reduction in the dose of captopril still exerts preventive benefit in this model without good control of the blood pressure, or limitation of proteinuria. According to classic radiobiology, an irradiated organ fails when its irradiated constituent cells die in the cell division that follows irradiation. Parenthetically, this paradigm for normal tissue injury could well explain the delay between irradiation and the occurrence of radiation nephropathy—cell division rates are low in normal kidney. AII is a known renal cell growth facilitator. The protective effect of ACE inhibitors or AII blockers could then derive, at least in part, from delay or inhibition of renal cell proliferation. Figure 7 shows data that support this hypothesis. The cell proliferation that occurs in the first few weeks of radiation nephropathy is shown. Preventive use of the AII blocker L,158-809 significantly attenuates the tubular, but not the glomerular component, of this proliferative response. It is not known what type of tubular cell is implicated. These data also support the notion that tubular cell injury has

greater importance than does glomerular, at least in this model of radiation nephropathy.

In summary, although the precise pathogenic mechanisms and/or mediators involved in radiation nephropathy remain under active investigation, radiation nephropathy is no longer viewed as inevitable, progressive, and untreatable. Radiation-induced injury involves complex and dynamic interactions between glomerular, tubular, and interstitial cells (Fig 8). These serve both as autocrine and as paracrine, if not endocrine, targets of biologic mediators that mediate nephron injury and repair. AII is clearly a predominant player in this process; however, other factors, including nitric oxide, TGF-β, and PAI-1 appear to be involved. A growing body of evidence supports a causative role of oxidative stress in fibrogenesis.[108] Antioxidants, particularly superoxide dismutase, have proven effective both in terms of inhibiting and, indeed, in reversing established fibrosis.[109] Given the recent demonstration of an apparent chronic, persistent, oxidative stress in the irradiated kidney,[100] there also might be potential for antioxidant-mediated modulation of radiation nephropathy. Future research should lead ultimately to the selection of a multipronged therapeutic approach targeted to the multicellular response of the irradiated kidney.

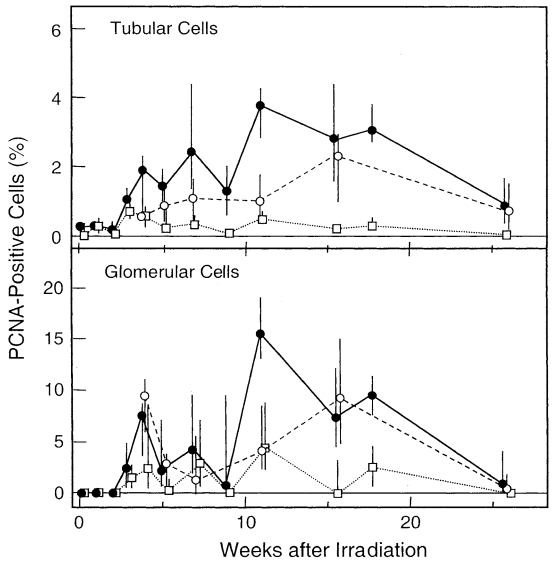

Fig 7. Time course of renal proliferation rates (PCNA labeling) of renal tubular and glomerular cells in rats given 17 Gy alone (●) or 17 Gy plus an AII receptor blocker (○) compared with unirradiated age-matched controls (□). Data are shown as median PCNA staining rats with 20% to 80% ranges. Reproduced with permission.[69]

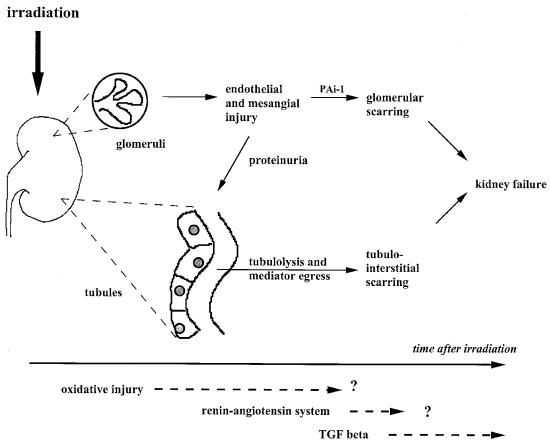

Fig 8. Simplified schema of radiation nephropathy. Sufficient ionizing radiation injures most or all components of the kidney. Glomerular injury is chronologically first, and involves at least its endothelium and mesangium, with evolution to glomerular scarring. The involvement of the fibrin-fibrinolytic system, via PAI-1, is shown, although other mediators could be active. Expression of tubular injury appears to occur somewhat later, even if it is set in motion at the same time as the glomerular injury. Oxidative injury, here as in glomeruli, could play a mechanistic role. Denuded tubules could allow interstitial entry to mediators that escape from injured glomeruli. Local mediator expression, such as TGFβ1, could be key in creating tubulointerstitial scarring. The time of system or mediator involvement is variable, as shown by the variable length of the arrows for oxidative injury, the renin-AII system, or the involvement of TGFβ1.

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