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The pathogenesis of osteodystrophy after renal transplantation as detected by early alterations in bone remodeling

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The pathogenesis of osteodystrophy after renal transplantation as detected by early alterations in bone remodeling.

Background. Loss of bone mass after transplantation begins in the early periods after transplantations and may persist for several years, even in patients with normal renal function. While the pathogenesis of these abnormalities is still unclear, several studies suggest that preexisting bone disease, glucocorticoid therapy, and alterations in phosphate metabolism may play important roles. Recent studies indicate that osteoblast apoptosis and impaired osteoblastogenesis play important roles in the pathogenesis of glucocorticoid-induced osteoporosis.

Objectives. To examine the early alterations in osteoblast number and surfaces during the period following renal transplantation.

Methods. Twenty patients with a mean age of 36.5 ± 12 years were subjected to bone biopsy 22 to 160 days after renal transplantation. In 12 patients, a control biopsy was performed on the day of transplantation. Bone sections were evaluated by histomorphometric analysis and cell DNA fragmentation by the methods of terminal deoxynucleotidyl transferase-mediated uridine triphosphate nick end labeling (TUNEL), using immunoperoxidase and direct immunofluorescence techniques.

Results. The main alterations in posttransplant biopsies were a decrease in osteoid and osteoblast surfaces, adjusted bone formation rate, and prolonged mineralization lag time. Peritrabecular fibrosis was markedly decreased. None of the pretransplant biopsies revealed osteoblast apoptosis. In contrast, TUNEL-positive cells in the proximity of osteoid seams or in the medullary space were observed in nine posttransplant biopsies of which four had mixed bone disease, two had adynamic bone disease, one had osteomalacia, one had osteitis fibrosa, and one had mild hyperparathyroid bone disease. Osteoblast number in posttransplant biopsies with apoptosis was lower as compared with posttransplant biopsies without apoptosis. In addition, most of them showed a marked shift toward quiescence from the cuboidal morphology of active osteoblasts. Serum phosphorus levels were lower in patients showing osteoblast apoptosis and correlated positively with osteoblast number and negatively with the number of apoptotic osteoblasts. In addition, posttransplant osteoblast surface correlated positively with parathyroid hormone (PTH) levels and negatively with glucocorticoid cumulative dose.

Conclusion. The data suggest that impaired osteoblastogenesis and early osteoblast apoptosis may play important roles in the pathogenesis of posttransplant osteoporosis. The possible mechanisms involved in the pathogenesis of theses alterations include posttransplant hypophosphatemia, the use of glucocorticoids, and the preexisting bone disease. PTH seems to have a protective effect by preserving osteoblast survival.

Key words: renal transplantation, bone histopathology, parathyroid hormone, renal osteodystrophy, transplant bone disease, bone cell apoptosis, osteoblast apoptosis, posttransplant bone disease.

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Bone mass loss after transplantation is a well-described phenomenon that starts in the early periods after transplantations and may persist for several years, even in patients with normal renal function [1–6]. While the pathogenic mechanisms of these abnormalities are still unclear, several studies suggest that preexisting bone diseases, immunosuppressive drugs [1–6], persistently elevated levels of parathyroid hormone (PTH) [6–9] and alterations of phosphate metabolism [10, 11] may play important roles. From the majority of published studies, the main bone alterations in bone remodeling after renal transplantation are changes (decrease) in bone formation in the face of persistent bone resorption. This produces an imbalance in remodeling favoring resorption [4–6]. Therefore, it is possible that the defective bone formation may be a consequence of either alterations in osteoblast function, decreased generation, or increased osteoblast death rates. There is evidence suggesting that under normal conditions, osteoblast number at the beginning of a remodeling period differs markedly at the end of the same cycle, suggesting that an important number of cells undergo apoptosis [12]. Furthermore, recent studies in mice indicate that glucocorticoids promote osteoblast and osteocyte apoptosis and inhibit osteoblastogenesis, resulting in the defective bone formation observed in glucocorticoid-induced osteoporosis [13]. Conversely, it has been shown that in mice with osteope-

nia due to defective osteoblastogenesis, PTH increases bone formation by preventing osteoblast apoptosis [14]. Based on these observations, the present study was designed to examine the possible role of an early increase in osteoblast apoptosis and alterations in osteoblastogenesis, as well as the influence of preexisting bone disease in the histomorphometric alterations in bone after transplantation. The studies were performed shortly after renal transplantation, a period in which patients are exposed to high doses of glucocorticoids and in which some of the preexisting alterations of bone metabolism may still be present.

METHODS

Patients

Twenty patients participated in a prospective study to evaluate the early bone alterations occurring after renal transplantation. All patients signed informed consent. Exclusion criteria were persistent posttransplant renal insufficiency requiring dialysis and sepsis. There were 12 men and eight women with a mean age of 36.5 ± 12 years. Patients had been on dialysis prior to transplantation for 21.4 ± 7.3 months. The underlying kidney diseases leading to end-stage renal diseases were chronic glomerulonephritis ($N = 9$), nephrosclerosis ($N = 2$), polycystic kidney disease ($N = 2$), urolithiasis ($N = 1$), and unknown ($N = 6$). Posttransplant immunosuppressive therapy consisted of glucocorticoids, cyclosporine, and mycophenolate mofetil. Acute rejection occurred in four patients and was treated with intravenous methylprenisolone pulse for 3 consecutive days. None of the patients received glucocorticoids, immunosuppressant drugs, vitamin D or analogs, bisphosphonates, or other drugs associated with alterations of bone metabolism during the dialysis period.

Biochemical determinations

Serum samples were obtained at time of bone biopsies for biochemical determinations. Calcium, phosphorus, creatinine, and total alkaline phosphatase were determined by routine laboratory techniques. Bone-specific alkaline phosphatase was assessed by enzyme-linked immunosorbent assay (ELISA) (Metra Biosystem, Inc., Mountain View, CA, USA). Intact PTH levels were determined by immunoradiometric analysis (Allegro™; Nichols Institute, San Juan Capistrano, CA, USA). Serum osteocalcin was determined using an immunoradiometric assay (Nichols Institute).

Bone biopsies and bone histology

Transiliac bone biopsies were performed at the anterior iliac crest using a Bordier needle. In 11 patients, a control biopsy was performed on the same day of transplantation. In the remaining nine patients, receiving kidney from cadaveric donors, only a posttransplant biopsy was performed. In 17 patients, the time elapsed from the date of transplantation to bone biopsy ranged from 22 to 60 days (35 ± 14 days), and 90 to 160 days (135.6 ± 31 days) in the remaining three patients. Double tetracycline labeling was obtained in 11 posttransplant biopsies.

Undecalcified bone specimens were fixed in ethanol, included in methyl methacrylate and cut for histomorphologic examination. Sections were stained by the Masson-Goldner trichromic method [15]. Aluminum was stained by the aurin tricarboxylic acid method [16], and iron by the Perls' method [17]. Histomorphometric analysis was performed using light microscopy, a Merz Schaenk reticle, and an eyepiece micrometer [18]. Static and dynamic parameters of bone structure are expressed according to the American Society of Bone and Mineral Research (ASMBR) nomenclature committee [19]. The normal reference values used [20–28] are in general agreement with data obtained for normal Latin American population [abstract; Jorgetti V et al, *Bone* 23 (Suppl 5): S476, 1998].

Detection of apoptosis

Bone sections were examined for cell DNA fragmentation by the methods of terminal deoxynucleotidyl transferase-mediated uridine triphosphate nick end labeling (TUNEL) using both immunoperoxidase and direct immunofluorescence techniques. For immunoperoxidase studies, undecalcified sections were deplasticized and treated with 5 μg/mL proteinase K for 15 minutes at room temperature. Endogenous peroxidase was quenched with 3% H_2O_2 in phosphate-buffered saline (PBS) for 5 minutes. Terminal deoxynucleotidyl transferase (TdT) (Apoptag Plus Peroxidase In Situ Apoptosis Detection Kit, Intergen Co., New York, NY, USA) and digoxigenin-labeled uridine triphosphate (dUTP) were added and sections were incubated for 1 hour at 37°C. TUNEL signal was detected with peroxidase-labeled antidigoxigenin antibody and developed with diaminobenzidine. Sections were counterstained with methyl green.

For direct immunofluorescence studies, deplasticized bone sections were rehydrated and treated with 20 μg/mL proteinase K in PBS for 15 minutes at room temperature. Sections were rinsed twice with PBS. Equilibration buffer was added directly to the specimens. Samples were labeled according to the instruction of the commercial kit (Apoptag Fluoroscein Direct In Situ Apoptosis Detection Kit, Intergen Co., New York, NY, USA). Briefly, tissue sections were covered with the TUNEL reaction mixture and incubated in a dark humidified chamber at 37°C for 60 minutes. The slides were rinsed twice with stop/wash buffer for 20 minutes and mounted with glass coverslip and mounting media. The slides were viewed using a fluorescent microscope.

Weaned rat mammary tissue, supplied by the manufac-

Table 1. Serum biochemical and hormonal parameters in 20 patients at the times of pre- and posttransplant bone biopsies

Parameter	Mean		*P* value
	Pretransplant	Posttransplant	
Calcium *mg/dL*	9.2 ± 0.8	8.9 ± 0.7	NS
Phosphorus *mg/dL*	5.1 ± 1.4	2.8 ± 0.25	<0.01
Total alkaline phosphatase *U/dL*	151 ± 115	139 ± 46	NS
Bone alkaline phosphatase	76 ± 34	41 ± 28	NS
Osteocalcin *nmol/mL*	159 ± 25	103 ± 88	NS
Parathyroid hormone *pg/mL*	593 ± 760	174 ± 240	<0.05

Results are expressed as mean ± SD.

turer, was used as a positive control. Negative controls were made by omitting the transferase from the reaction mixture [23].

Statistical analysis

Results are expressed as mean ± standard deviation. Statistical analysis was performed by Student *t* test and multiple regression analysis. $P < 0.05$ was considered significant.

RESULTS

Biochemical determinations

The results of blood biochemical analysis of the 20 patients are shown in Table 1. There were no statistical significant differences in calcium, total alkaline phosphatase, bone specific alkaline phosphatase, and osteocalcin pre- and posttransplantation. However, phosphorus (5.1 ± 1.4 mg/dL vs. 2.8 ± 0.25 mg/dL, $P < 0.01$) and PTH (593 ± 760 pg/mL vs. 174 ± 240 pg/mL, $P < 0.05$) decreased significantly after transplantation. Serum creatinine at the time of posttransplant biopsies was within the normal range (1.3 ± 0.2 mg/dL).

Bone histology

The histologic diagnosis in the whole group of patients were as follows: five had osteitis fibrosa, two had mild hyperparathyroidism, six had mixed bone disease, three had adynamic bone disorder, two had osteoporosis, one had osteomalacia, and one had normal bone histology. Aluminum in more than 30% of bone trabeculae was found only in two patients, both of them with mixed bone disease.

Histomorphometric parameters. Table 2 shows the histomorphometric parameters in pre- and posttransplant bone biopsies in the whole group of patient compared with normal reference values [20–22]. Trabecular bone volume was within the normal range and remained unchanged in this early period following transplantation. Osteoid volume, osteoid thickness, resorption surface, and osteoclast surface were above the normal range in pretransplant biopsies, remaining unchanged after transplantation. In contrast, osteoid and osteoblast surfaces were elevated in pretransplant biopsies, but decreased significantly after transplantation.

Table 2. Histomorphometric parameters in pre- and posttransplant bone biopsies

Parameter	Pretransplant	Posttransplant	Normal reference values[a]
Bone volume/tissue volume %	24.7 ± 4.8	22.8 ± 5.1	21.2 ± 5.1
Osteoid volume/ tissue volume %	6.7 ± 4.03	6.01 ± 4.4	2.7 ± 1.8
Osteoid surface/ bone surface %	46.2 ± 16.9[b]	32.7 ± 15.0	14.2 ± 7.7
Erosion surface/ bone surface %	13.1 ± 4.3	11.8 ± 5.5	3.6 ± 1.1
Osteoclast surface/ bone surface %	8.0 ± 3.5	5.8 ± 3.3	0.6 ± 0.1
Osteoblast surface/ bone surface %	22.1 ± 9.4[c]	15.8 ± 10.9	4.9 ± 1.4
Osteoid thickness *μm*	15.3 ± 6.3	15.3 ± 5.7	10.0 ± 1.8
Osteoblast number/ tissue surface *mm²*	0.33 ± 0.17	0.13 ± 0.11	
Fibrosis surface/ bone surface %	16.1 ± 29.2	6.9 ± 18.1	0
Mineralizing surface/ bone surface %		12.6 ± 2.9	18 ± 8
Mineral apposition rate *μ/day*		0.7 ± 0.14	0.65 ± 0.1
Bone formation rate/ bone surface $\mu^3/\mu^2/day$		0.08 ± 0.04	0.13 ± 0.07
Adjusted bone formation rate $\mu^3/\mu^2/day$		0.29 ± 0.15	0.5 ± 0.2
Mineralization lag time *days*		57.3 ± 48	21.3 ± 2

Results are expressed as mean ± SD.
[a]Data from references [19–21]
[b]$P < 0.01$
[c]$P < 0.05$

To further examine the effects of transplantation on bone histomorphometry, we compared static parameters in paired biopsies performed in 11 patients at the time of transplantation and 60 ± 38 days after transplantation. As shown in Figure 1, osteoid and osteoblast surfaces decreased significantly after transplantation. In addition, peritrabecular fibrosis decreased markedly or disappeared. All other posttransplant parameters did not show significant changes compared with pretransplant biopsies. Similar results were obtained when histomorphometric parameters in pretransplant biopsies were compared with those of the whole group of 20 patients studied posttransplant, but, as expected, paired biopsies corrected for the variable forms of renal osteodystrophy, increasing the significance of the changes due to transplantation.

Bone dynamic histomorphometric parameters were determined in 11 patients that underwent posttransplant double tetracycline labeling (four patients had mixed bone disease, two had adynamic bone disease, three had

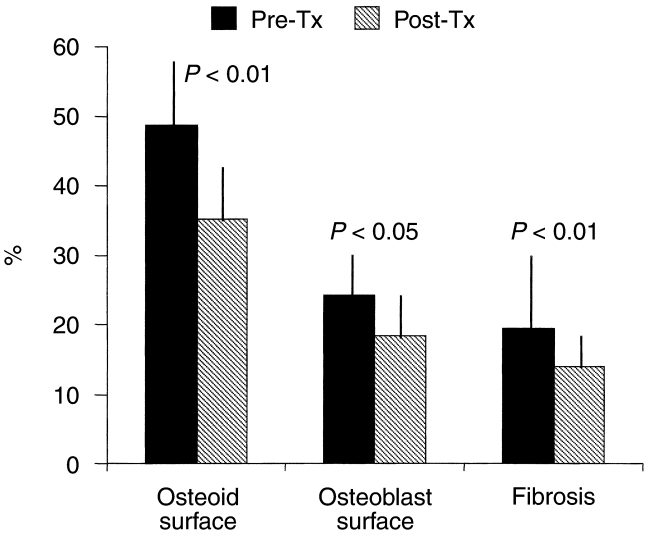

Fig. 1. Comparison of static histomorphometric parameters of 11 paired bone biopsies performed at the times of transplantation (pre-Tx) and 38 ± 17 days after transplantation (post-Tx). The numbers are expressed as mean ± standard deviation.

hyperparathyroid bone disease, one had osteoporosis, and one had normal bone histology). As shown in Table 2, all dynamic parameters were below the normal reference values. However, the most prominent changes were a reduction in adjusted bone formation rate and a markedly prolonged mineralization lag time compatible with the changes in osteoblast and osteoid surface shown in Figure 1.

Osteoblast apoptosis. All pre- and posttransplant biopsies were examined for apoptosis using the method of TUNEL. None of the pretransplant biopsies showed bone cell apoptosis by these techniques. In contrast, as shown in Table 3, TUNEL-positive cells were observed in nine posttransplant biopsies, of which four had mixed bone disease, two had adynamic bone disease, one had osteomalacia, one had osteitis fibrosa, and one had mild hyperparathyroid bone disease (Table 3). This phenomenon appeared to be independent of the time elapsed between transplantation and posttransplant biopsy. Figure 2 shows representative preparations of bone biopsies with TUNEL-positive cells. TUNEL-positive osteoblasts were clearly observed in the proximity of osteoid seams (Fig. 2 A to C). TUNEL-positive osteocytes were observed in one of the biopsies from a patient with adynamic bone disease (Fig. 2D). In addition, in the majority of specimens, numerous apoptotic bodies were observed free or within macrophages in the bone marrow space. Osteoclast apoptosis was not observed.

As shown in Figure 3A, the total osteoblast number per tissue surface in posttransplant biopsies with apoptosis was lower as compared with those without apoptosis ($0.15 \pm 0.09/mm^2$ vs. $0.35 \pm 0.17/mm^2$, $P < 0.01$). In addition, there was a change in osteoblast morphology, demonstrating a marked shift toward quiescence or inactive form from the cuboidal morphology of active osteoblasts, even in the presence of elevated osteoid thickness in many patients, suggesting defective mineralization. Figure 3B shows a highly significant correlation between total osteoblast number and active osteoblasts. Thus, patients with lower osteoblast number had also a lesser number of active osteoblasts. In favor of these observations, patients with osteoblast apoptosis showed a significant decrease in posttransplant serum osteocalcin levels (Fig. 4).

Table 3. Posttransplant osteoblast apoptosis according with the type of bone disease

Bone disease	Total	Apoptosis	No apoptosis
Adynamic bone disease	3	2	1
Osteomalacia	1	1	0
Mixed bone disease	6	4	2
Mild hyperparathyroidism	2	1	1
Osteitis fibrosa	5	1	4
Osteoporosis	2	0	2
Normal histomorphometry	1	0	1
Total	20	9	11

Osteoblast apoptosis and serum phosphorus. To examine the possible causes of increased posttransplant apoptosis, we compared the pre- and posttransplant serum biochemical parameters in patients with or without osteoblast apoptosis. An interesting observation was an association between posttransplant osteoblast apoptosis and serum phosphorus (Fig. 5A). Thus, serum phosphorus levels were significantly lower in patients with biopsies showing osteoblast apoptosis (2.3 ± 0.4 mg/dL vs. 3.4 ± 0.8 mg/dL in apoptosis-negative biopsies, $P < 0.01$).

To examine a possible effect of phosphorus on osteoblast genesis or survival, we correlated osteoblast number and serum phosphorus levels in all patients studied. As shown in Figure 5B, there was a highly significant correlation between posttransplant osteoblast number and serum phosphorus (r, 0.621; $P < 0.01$). Furthermore, the number of posttransplant apoptotic cells in the biopsies correlated negatively with posttransplant serum phosphorus (r, -0.641; $P < 0.01$).

In agreement with previous studies [11], the changes in serum phosphorus were independent of PTH, since posttransplant PTH levels in both groups were similar (105.5 ± 47.4 pg/mL vs. 130.3 ± 119 pg/mL, *NS*) and there was no correlation between the serum hormone levels and phosphorus pre- or posttransplantation (Fig. 6).

Effect of PTH and glucocorticoids. As a means to evaluate the possible effect of PTH on posttransplant alterations in bone remodeling, we examined the correlation of pre- or posttransplant levels of the hormone with the different histomorphometric parameters in posttransplant bone specimens. The main findings were a

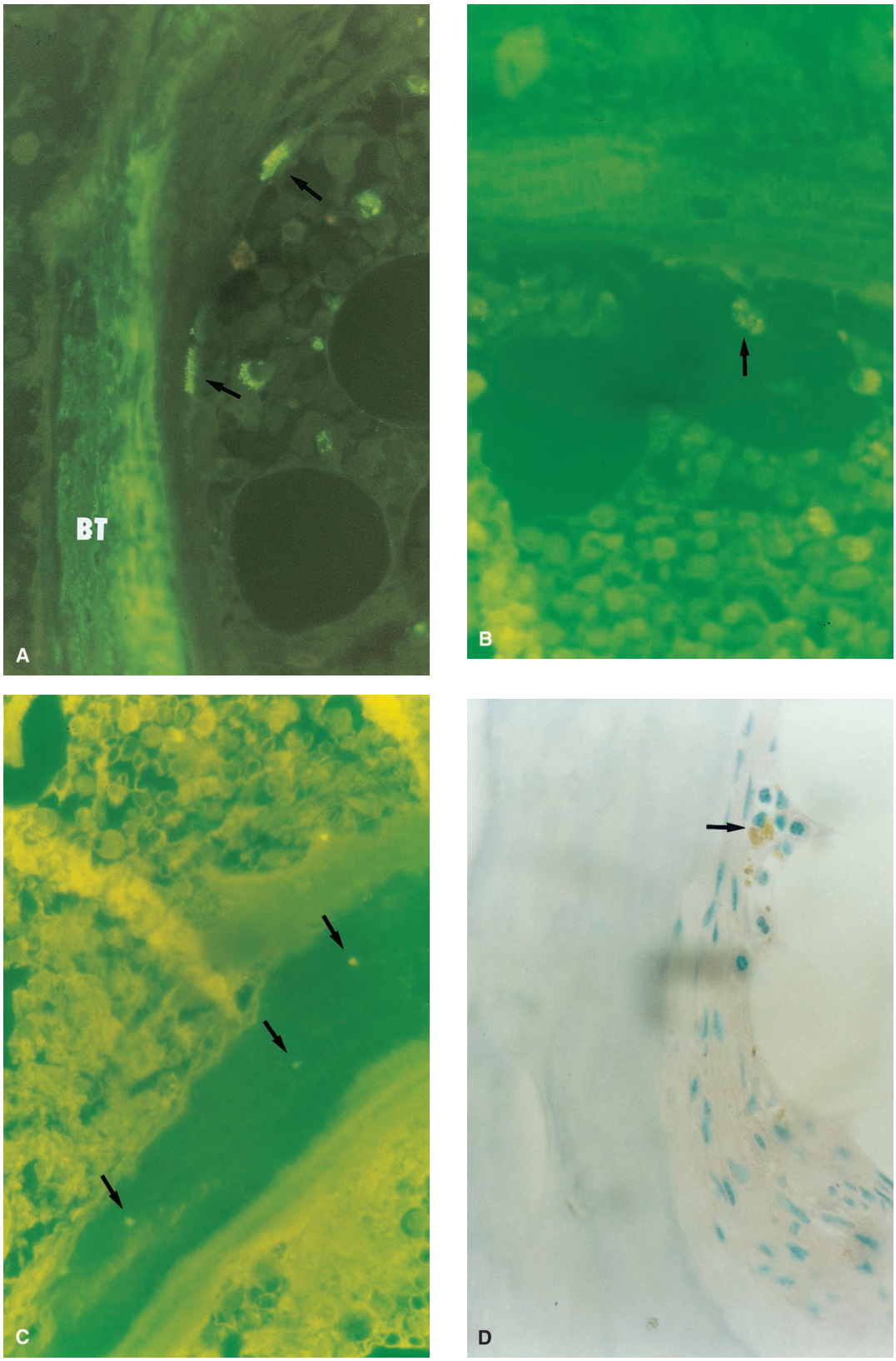

Fig. 2. Representative microphotograph of bone biopsies showing cell apoptosis by the method of terminal deoxynucleotidyl transferase-mediated uridine triphosphate nick end labeling (TUNEL). (*A*) Direct immunofluorescence staining of a biopsy of a patient with mixed bone disease showing TUNEL-positive osteoblasts. (*B*) Direct immunofluorescence staining of a biopsy from a patient with osteomalacia showing apoptotic bodies in the proximity of osteoid seams. (*C*) Direct immunofluorescence staining showing apoptotic osteocytes. (*D*) Immunoperoxidase staining of a biopsy of a patient with osteitis fibrosa showing TUNEL-positive cells in the proximity of osteoid seams. BT is bone trabeculae.

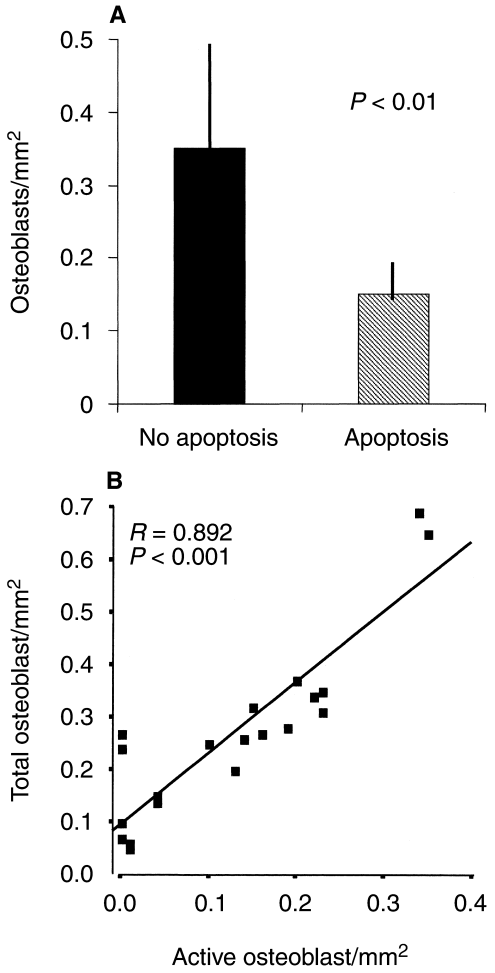

Fig. 3. (*A*) Osteoblast number per tissue surface in bone biopsies of patients showing osteoblast apoptosis (Apoptosis, $N = 9$) compared with those without evident apoptosis (No apoptosis, $N = 11$). The numbers are expressed as mean ± standard deviation. (*B*) Correlation between the total number and the number of active osteoblasts as determined by the typical cuboidal morphology.

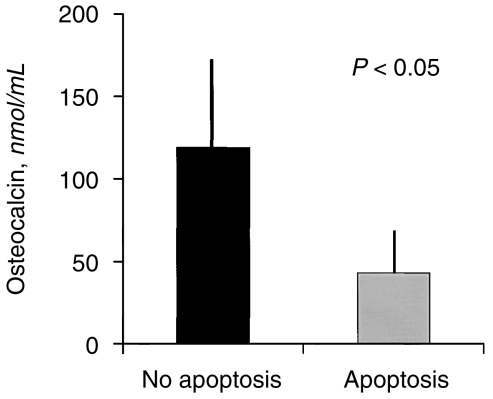

Fig. 4. Posttransplant serum osteocalcin levels in patients showing apoptosis compared with patients without osteoblast apoptosis in posttransplant bone biopsies. The numbers are expressed as mean ± standard deviation.

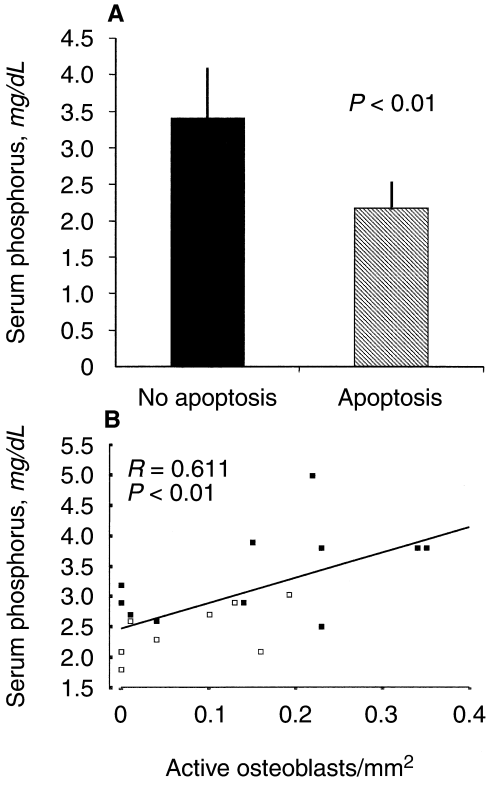

Fig. 5. (*A*) Posttransplant serum phosphorus levels in patients showing apoptosis compared with patients without osteoblast apoptosis in posttransplant bone biopsies. (*B*) Relationship between serum phosphorus and apoptosis of osteoblasts.

highly significant positive correlation between pretransplant PTH levels and posttransplant osteoblast surface (Fig. 7A), as well as a correlation between posttransplant PTH and osteoblast surface. Thus, patients with higher pretransplant or posttransplant PTH levels showed a larger osteoblast surface than those with low PTH. In contrast, there was a negative correlation between glucocorticoid cumulative dose and posttransplant osteoblast surface (Fig. 8).

There was no correlation between cyclosporine or mycophenolate mofetil cumulative doses and the histomorphometric alterations observed in posttransplant biopsies or serum biochemical changes in our patients.

DISCUSSION

Previous studies suggest that the bone loss observed after renal transplantation may start very early and persist for years [1–4]. However, the mechanisms remain unclear. Therefore, the present studies were designed to examine the histologic bone changes occurring in the early period that follows renal transplantation. The results demonstrate early posttransplant apoptosis of osteoblasts and a decrease in osteoblast number and surface, as well as a decrease in bone formation rate and a

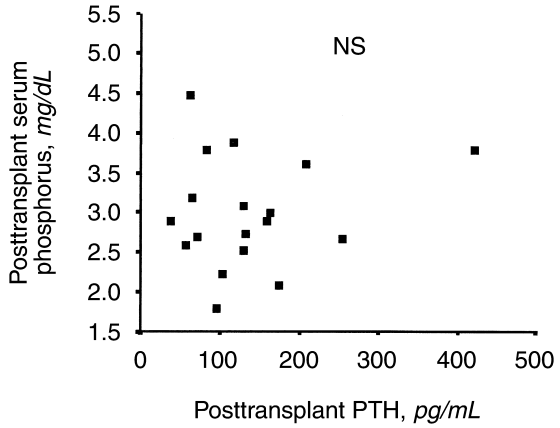

Fig. 6. Correlation between posttransplant serum phosphorus and parathyroid hormone (PTH) levels.

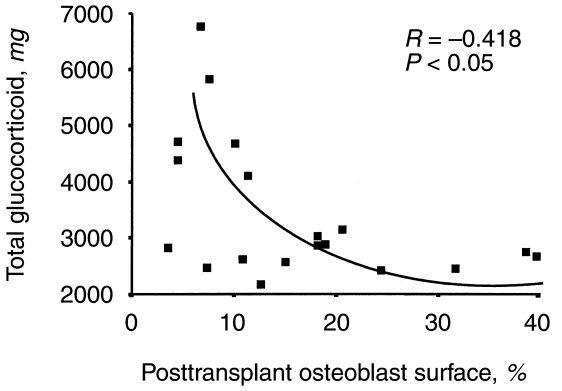

Fig. 8. Correlation between the posttransplant cumulative dose of glucocorticoids and osteoblast surface.

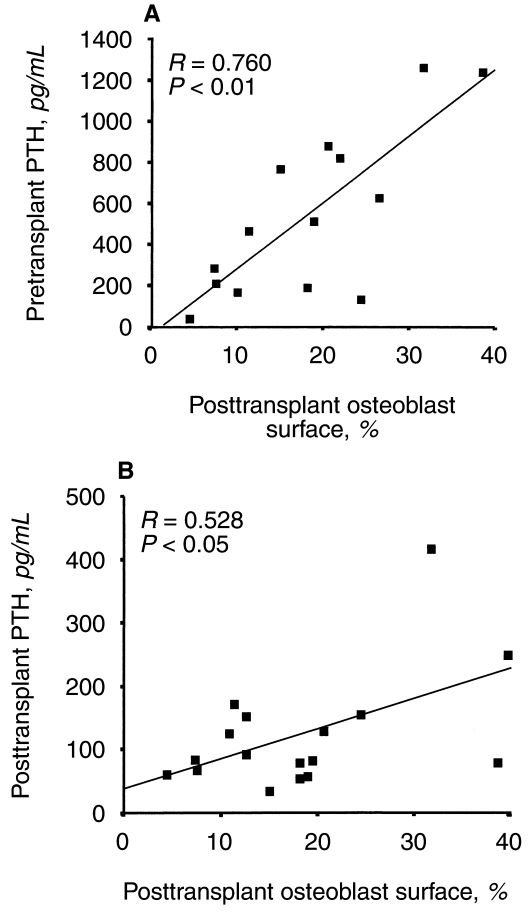

Fig. 7. Correlation between serum parathyroid hormones (PTH) levels and osteoblast surface. (*A*) Correlation between pretransplant serum PTH levels and posttransplant osteoblast surface. (*B*) Correlation between posttransplant serum PTH levels and posttransplant osteoblast surface.

delayed mineralization lag time, suggesting that impaired osteoblast number and function may play a role in the pathogenesis of posttransplant bone disease. Several factors, including the preexisting bone disorder, alterations in mineral metabolism, as well as the use of glucocorticoids, seem to play a role in the pathogenic mechanisms leading to these alterations.

Pretransplant biopsies showed different histologic abnormalities, including osteitis fibrosa, mixed and adynamic bone disease, osteomalacia, and osteoporosis. Most of the patients showed a decrease in posttransplant osteoblast surfaces and number independent of the predominant bone disease. Thus, reduced osteoblast surfaces were observed in patients with low bone turnover, as well as in those with high bone turnover. A striking finding was a marked tendency to change from the typical cuboidal osteoblast morphology to the quiescent status, indicating an early decrease in osteoblast activity after transplantation. Indeed, most osteoblasts looked quiescent, even in presence of increased osteoid surface and thickness, resulting in a decreased bone formation rates and prolonged mineralization lag time in the majority of patients who underwent double tetracycline labeling after transplantation. These alterations in bone turnover are similar to those observed in patients with long-term renal transplantation [4], including the development of generalized or focal osteomalacia in many of them [6], suggesting that the pathogenic mechanisms involved start operating early and are maintained for years after transplantation.

Recent studies have demonstrated that osteoblasts, in addition to becoming lining cells and osteocytes, undergo apoptosis with a frequency sufficient to explain the difference in the number of osteoblasts in remodeling bone [24]. Furthermore, it has been demonstrated that increased apoptosis and reduced osteoblastogenesis may be one of the main mechanisms of glucocorticoid-induced osteoporosis. In our study, none of the pretransplant biopsies examined showed osteoblast apoptosis. However, nine of the patients studied showed osteoblast apoptosis in the early period after transplantation. These patients also showed lower osteoblast surface and number. There were no differences in posttransplant time between patients showing apoptosis compared with

those in which this phenomenon could not be demonstrated, indicating that the increase in apoptosis in these patients cannot be attributed to the time elapsed after transplantation. In contrast, the preexisting bone disease seems to play a role, since osteoblast apoptosis was more frequently observed in patients with adynamic bone disease, osteomalacia, and mixed bone disease, whereas it was relatively rare in patients with high bone turnover. It is important to mention that, although apoptosis is a natural phenomenon, it is difficult to demonstrate since apoptotic bodies are short lasting [24]. Therefore, the observation of apoptotic cells or bodies in some posttransplant biopsies and not in pretransplant specimens may indicate either that there is an increase in the time span of apoptotic bodies or that there occurs an increase in the proportion of cells that undergo apoptosis. This last possibility is in accordance with the observation that patients showing increased apoptosis had also lower osteoblast surface and number.

Several mechanisms may be involved in the increased apoptosis after renal transplantation. We found that cumulative dose of glucocorticoids correlated negatively with posttransplant osteoblast surface. As mentioned above, recent studies strongly suggest that osteoblast and osteocyte apoptosis, and inhibition of osteoblastogenesis may play a central role in glucocorticoid-induced osteoporosis [13–24]. Since bone biopsies in our patients were performed early after transplantation, a period of maximal glucocorticoid dose, it seems possible that glucocorticoids may play a role in the increase of apoptosis observed in many of our patients. In contrast, we did not find any correlation between cyclosporine and the alterations of bone histology posttransplantation.

Patients showing posttransplant apoptosis had significantly lower serum phosphorus levels compared with those without apoptosis. Furthermore, posttransplant serum phosphorus correlated negatively with the number of apoptotic osteoblasts. In addition, there was a highly significant correlation between osteoblast number and the percentage of active osteoblasts, suggesting a role of phosphorus in the pathogenic mechanisms leading to posttransplant bone disease. Indeed, hypophosphatemia has been associated with severe alterations in bone turnover that include a decrease in osteoblast activity leading to rickets and osteomalacia [26–28]. Our results strongly suggest a causative effect of hypophosphatemia on the decrease in bone formation and the increased mineralization lag time posttransplant. Since posttransplant hypophosphatemia may be due to high levels of phosphatonin, these results call into question the effects of phosphatonin on osteoblast function.

Hypophosphatemia, a frequent disorder after renal transplantation [10, 11, 29, 30], may be contributed to by inappropriate phosphaturia as a consequence of persistently elevated PTH levels, glucocorticoids, and relatively low levels of 1,25(OH)2 vitamin D_3. However, recent studies strongly suggest the presence of a circulating humoral factor (phosphatonin) that induces phosphaturia independent of PTH [31, 32]. The fact that we did not find correlation between serum phosphorus levels with PTH or glucocorticoid cumulative dose suggests that this phosphaturic humoral factor could be involved in the posttransplant hypophosphatemia observed in our patients. Indeed, recent studies demonstrate that serum obtained from patients early after transplantation inhibits Na/Pi activity in proximal tubular opossum kidney (OK) cells, while the expression of Na/Pi mRNA and protein increases, independent of PTH. These effects were also induced by sera from patients with advanced chronic renal failure, but not when cells were incubated with late posttransplant sera, suggesting that persistence of a "phosphatonin" factor in the early posttransplant period would be responsible for the hyperphosphaturia and hypophosphatemia observed after transplantation. While the nature of the "phosphatonins" in these patients has not been elucidated, recent studies have described the potential identity of "phosphatonins" in other disorders characterized by hyperphosphaturia and hypophosphatemia, such as oncogenic osteomalacia (OOM), X-linked hypophosphatemic rickets (XLHR), and autosomic-dominant hypophosphatemic rickets (ADHR) [33–35]. One of them, fibroblast growth factor-23 (FGF-23), induces phosphaturia and hypophosphatemia when administered to normal mice. Furthermore, long-term exposure to FGF-23 by implantation of Chinese hamster ovary stably expressing FGF-23 into nude mice induced hypophosphatemia and severe osteomalacia [33]. The fact that FGF-23 also decreases 1α-hydroxylase messenger mRNA [33, 34] provides an additional explanation for the defect of mineralization observed in these situations. Furthermore, a direct effect of FGF-23 on osteoblasts was suggested by the findings of growth retardation in knockout mice, and the expression of phosphate-regulating gene with homologies to endopeptidase on the X chromosome (PHEX) and FGF-23 in these cells [36]. Another phosphaturic factor, frizzled-related protein 4 (FRP4), isolated from OOM tumors, has been associated with expression of apoptosis-related genes [37], but a direct induction of apoptosis has not been demonstrated. Therefore, although our studies suggest that "phosphatonins" may play a role in the pathogenesis of hypophosphatemia in our patients, they may provide a link between hypophosphatemia and alterations in osteoblastogenesis or osteoblast apoptosis following transplantation.

Finally, an interesting finding in posttransplant biopsies was a positive correlation between osteoblast surface and the serum levels of pre- and posttransplant PTH, suggesting an important role of the hormone in preserving osteoblast number and activity after transplantation.

Indeed, previous studies by Jilka et al [14] indicate that in mice, PTH increases the life span of mature osteoblasts by preventing their apoptosis. These findings are also in agreement with the fact that posttransplant apoptosis was rare in patients with pre-transplant secondary hyperparathyroidism.

CONCLUSION

The present studies demonstrate that the posttransplant bone disease starts very early after transplantation, probably as a consequence of early apoptosis of osteoblasts and decreased osteoblastogenesis. The possible mechanisms involved in the pathogenesis of theses alterations include posttransplant hypophosphatemia, the use of glucocorticoids, and the preexisting bone disease. PTH seems to play a protective effect by preserving osteoblast survival.

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