Cell Biology of Parathyroid Gland Hyperplasia in Chronic Renal Failure

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Secondary hyperparathyroidism is a well known feature of chronic renal failure. It is characterized by an increase in the synthesis and secretion of parathyroid hormone (PTH), mainly due to disturbances of calcium, phosphate, and vitamin D metabolism. Our understanding of the mechanisms by which the decrease in plasma calcium and calcitriol and the increase in plasma phosphate in advanced stages of chronic renal failure stimulate PTH secretion has greatly improved during the last decade. In addition to several long known indirect effects, they all have also been shown to act directly on the parathyroid cell, via a cell membrane receptor for calcium (1), a nuclear receptor for calcitriol (2), and a yet unknown cellular target for phosphate (3–5). Disturbances of receptor expression and binding, postreceptor signaling pathways, receptor-ligand complex action upon the pre-pro-PTH gene, and/or PTH mRNA stability and processing have been reported, which all may contribute to stimulate PTH synthesis and secretion (6–10).

In the past, the mechanisms of abnormal PTH release and action in uremic patients and animals have received much greater attention than the mechanisms underlying parathyroid hyperplasia. This can be explained by the remarkable progress made during recent years in understanding the function, regulation, and expression of the pre-pro-PTH gene and of PTH receptors in target tissues, as well as by the availability of reliable tools for the exploration of these processes. Knowledge of the regulation of cell cycle activity and cell death program is still more fragmentary. Although it has remarkably progressed recently, its application to parathyroid gland tissue has remained modest.

One of the reasons is that this endocrine organ belongs to the class of low turnover, discontinuously replicating tissues, which are composed of cells with a long life span, as is the case for many, if not most, hormonally active tissues (11). The analysis of the growth characteristics of such tissues is generally hampered by the absence of appropriate cell culture models. In contrast, for tissues with a high turnover such as the skin, intestinal epithelium, bone marrow, and gonads, which have a short life span and are continuously replaced from a population of self-replicating stem cells, numerous cell culture models have become available. This has greatly facilitated the study of their normal and pathologic growth characteristics.

Another reason for the limited attention devoted to parathyroid gland size in the past is the relatively low precision of available measurement techniques. It appears that at present, despite remarkable advances in the field of noninvasive imaging procedures during the past decade, the normal parathyroid gland remains one of the rare organs of the human body, if not the only one, which can still not be reliably visualized. Although various techniques have been developed for the localization of abnormal parathyroid glands, with sestamibi scintigraphy probably being the most accurate method available to date, methods that allow one to determine the size of all hyperplastic glands in secondary (2°) hyperparathyroidism are still lacking. High-resolution ultrasonography probably is the technique with the highest degree of sensitivity for this purpose, although its specificity for parathyroid tissue versus other nodular formations in the neck region is relatively low.

Normal Parathyroid Cell Growth and Apoptosis

Normal parathyroid cells are characterized by an extremely low turnover. Their mean life span has been estimated by Parfitt’s group, using the expression of cell cycle-associated nuclear antigen Ki-67, to be approximately 2 yr in adult rats (12) and 20 yr in adult humans based on post mortem determinations (13). The parathyroid tissue of the latter exhibited a geometric mean labeling index of 1.44 cells/10,000 cells. This means that not much more than 1 cell out of 10,000 is actually engaged at any time in the cell cycle for eventual replication. However, other groups found much higher mean labeling index numbers in normal human parathyroid glands, by one to two orders of magnitude, using the same or similar (proliferating cell nuclear antigen) method (14,15). The observed differences may be due to the above-mentioned technical difficulties to accurately measure cellular growth rates in slowly proliferating tissues. They may also be due to differing modes of tissue conservation at the time of sampling, as, for instance, postmortem versus in vivo sampling.

Under physiologic steady-state conditions, at least in adult life, the rate of parathyroid cell apoptosis should equal that of proliferation and therefore be comparably low. It should the-
oretically be measurable, if sufficiently sensitive techniques are available. However, the problem is that the identification of apoptotic numbers in extremely slow-growing tissues such as the parathyroid has proved to be no easy undertaking. Three research groups recently examined the rate of apoptosis in normal rats on their usual diets, as well as in normal rats that had been submitted to various nutritional stimuli of parathyroid tissue proliferation, such as feeding low calcium or high phosphate diets. These groups were unable to find evidence of programmed parathyroid cell death in normal or stimulated hyperplastic parathyroid tissue (12,16,17). One explanation for this failure could be a lack of sensitivity of the employed methods, especially when considering that apoptosis is a rather rapid process, compared with the process of cell division (12). However, all three groups used the sensitive DNA terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) technique. Another possibility is that the digestion methods used to prepare parathyroid tissue for the TUNEL technique were inappropriate. We found that protein digestion is extremely harmful to cryopreserved human parathyroid tissue. The failure of identifying any degree of apoptosis could thus be due to a problem of tissue preparation.

In humans, three groups including our own found relatively high apoptotic figures in normal parathyroid glands using the TUNEL technique (18–20), amounting to 1 to 5 cells/100 cells (Figure 1). These numbers must be considered excessive, again due to technical difficulties (21). In a more recent study in which we refined the preparation of normal human parathyroid tissue samples for analysis by the TUNEL technique, we found a much lower prevalence of apoptosis, with a mean frequency of approximately 1 apoptotic figure per 10,000 cells (22). This prevalence is compatible with that of Ki-67-positive cells engaged in the cell cycle (21).

Enhanced Parathyroid Cell Proliferation in Chronic Renal Failure

Relation between PTH Oversecretion and Parathyroid Hyperplasia

It has long been known that a chronic increase in PTH production, characteristic of primary as well as of secondary forms of hyperparathyroidism, goes along with an increase in parathyroid gland size (23–25). This is also true for most other endocrine organs, in which secretory overactivity is generally associated with hypertrophy and/or hyperplasia. However, the link between the two processes, albeit obvious from a pathologic and clinical point of view, is far from being well understood in mechanistic terms (see below). In the various forms of primary hyperparathyroidism, enhanced cell proliferation probably comes first and increased PTH secretion second, whereas in secondary forms of hyperparathyroidism, the opposite sequence of events occurs. However, a direct link between hormone secretion and hyperplasia is not obligatory. Dissociations between the two processes have been observed, at least under short-term experimental conditions, as has been recently shown in uremic rats (26).

PTH oversecretion with its negative effects on bone is one of the hallmarks of early chronic renal failure (27). It has been shown repeatedly in the rat that the increase in PTH secretion immediately after 5/6 nephrectomy is intimately associated with the development of parathyroid gland hyperplasia (4,17,28). In patients with incipient chronic renal failure, there are no surgical or histologic data of early parathyroid hyperplasia, for obvious reasons. Unfortunately, the recently introduced sestamibi scintigraphy, although highly accurate in the location of overtly enlarged, hyperactive glands, is unable to detect moderate, early increases of parathyroid gland size (unpublished observation).

Types and Rate of Accelerated Parathyroid Tissue Growth

The increase of parathyroid cell mass in the chronic uremic state is mainly due to enhanced parathyroid cell proliferation,
characterized by an increase in cell number (24,29). Cell hypertrophy appears to play only a minor role, if any. This is different from the increase in parathyroid gland volume, mainly due to cell hypertrophy, which has been observed in response to hypocalcemia or hyperphosphatemia in animals with normal renal function put on a calcium-deficient diet (29,30) or on a phosphate-rich regimen (12). In contrast, in an animal model in which normal rats were fed a diet poor in both calcium and vitamin D, parathyroid cell hyperplasia largely prevailed over hypertrophy (16). The latter model of a mixed calcium and vitamin D deficiency resembles more closely the uremic state than the former two pure calcium deficiency or phosphate overload models.

Whether hyperplasia is always slow to develop, as claimed by Parfitt (24), is uncertain. Differences in cell proliferation rate may again depend on the experimental model used, i.e., on the main underlying stimulus. Thus, the hyperplasia after the induction of renal failure appears to be a more rapid process than that induced by a phosphate-rich diet in animals with normal renal function, starting within days after a 5/6 reduction of nephron mass (4). The rapid development of increased parathyroid cell proliferation after total nephrectomy in the rat was reported in 1950 (31).

**Diffuse, Polyclonal Parathyroid Tissue Growth**

In initial stages of chronic renal failure, parathyroid cell proliferation appears to be diffuse and homogeneous, at least in the rat. The same is probably true for human patients, although direct evidence is lacking. In contrast, nodular formations develop within enlarged parathyroid glands in advanced stages of renal failure, mostly in chronic dialysis patients with severe 2° hyperparathyroidism (see below).

Estimations of the growth rate of hyperplastic parathyroid tissue in chronic renal failure have led to variable results. In rats on moderately high to high phosphate diets, parathyroid gland weight, protein content, and total DNA content per gland doubled and reached a plateau within 2 wk after the creation of uremia (4,32), and a three- to sixfold increase of parathyroid cells engaged in cell cycle was found (16,17). In uremic patients on dialysis treatment, largely discrepant proliferative figures have been found (Figure 1), varying by one to two orders of magnitude between studies and also from one parathyroid gland to the other (14,15) (unpublished results). Such differences may be explained, at least in part, by the heterogeneous nature of parathyroid tissue growth in patients with severe 2° hyperparathyroidism (see below). Nonrandom sampling, that is, selecting regions where more cells of interest are evident, may have contributed to higher estimates in some reports. Finally, another reason for falsely high values is use of flow cytometry, which is inaccurate in low turnover tissues.

**Mechanisms Involved in Diffuse-Type, Polyclonal Parathyroid Hyperplasia**

The mode of parathyroid cell proliferation is almost certainly polyclonal in initial stages of renal failure (23). At the molecular level, an increased expression of the immediate early gene c-myc has been suggested to be involved in the enhanced parathyroid tissue growth of uremic rats (33). Acidic fibroblast growth factor autocrine system also has been proposed as a mediator of calcium-regulated parathyroid cell growth in a clonal cell model (34). However, the cells derived from rat parathyroid glands, which were used for this experiment, failed to produce PTH. They instead produced parathyroid hormone-related peptide (PTHrp). Recently, PTHrp has again been suggested to be involved in the regulation of parathyroid tissue growth, based on the observation of a negative correlation between the expression of PTHrp and parathyroid cell proliferative activity in hyperplastic tissue removed from chronic hemodialysis patients (35). PTHrp enhances or inhibits cell proliferation, depending on cell type and also on basal rate of proliferation (36).

We have shown that the hyperplastic parathyroid tissue of uremic patients is characterized by de novo expression of transforming growth factor-α and strong expression of its receptor, epidermal growth factor receptor (37). It remains to be seen to what extent the altered tissue expression of these factors is instrumental in the pathogenesis of parathyroid gland hyperplasia or whether it is only a secondary phenomenon.

Table 1 summarizes reported increases or decreases in the expression of genes or their products potentially involved in 2° uremic hyperparathyroidism.

The factors proximal to the induction of these growth-stimulating mechanisms appear to be essentially the same as those involved in PTH hypersecretion, i.e., calcium, phosphate, and vitamin D.

**Role of Calcium Deficiency in Vivo**

Calcium deficiency, in the presence or absence of hypocalcemia, together with vitamin D deficiency or reduced generation of calcitriol, probably is the most important stimulus.
Silver’s group showed that calcium deprivation, together with vitamin D deficiency, greatly enhanced the rate of parathyroid cell proliferation in rats with chronic renal failure, using the cell cycle-linked proliferating cell nuclear antigen (16). A concomitant decrease in calcium-sensing receptor expression, as observed in parathyroid glands of uremic rats (38) and chronic dialysis patients (9,10), should theoretically enhance parathyroid tissue hyperplasia further. That this is so has been proven indirectly by the observation that the administration of the calcimimetic compound NPS R-568, a calcium-sensing receptor agonist, led to the suppression of parathyroid cell proliferation in rats with renal insufficiency (17).

Role of Vitamin D Deficiency in Vivo

A relative or absolute deficiency of vitamin D or its active metabolite calcitriol has also long been suspected as a possible cause of enhanced parathyroid cell growth, based on experimental findings in other tissues. The importance of vitamin D in the parathyroid hyperplasia of experimental uremia was first shown by Ritz’s group (29). These authors administered increasing doses of calcitriol to rats either at the time of inducing chronic renal failure or at a later time point, when uremia was already well established. They were able to prevent parathyroid cell proliferation entirely when calcitriol was given at the start of uremia, but not when given later. Fukagawa et al. showed that pharmacologic doses of calcitriol repressed c-myc expression in the parathyroid tissue of uremic rats and suggested that the hormone might suppress parathyroid hyperplasia by this pathway (33). In contrast, Naveh-Many et al. (16) failed to observe such an antiproliferative effect of calcitriol in parathyroid cells of uremic rats, but they administered the hormone for only three subsequent days. This short-term administration may not have been sufficient for an efficacious suppression of cell turnover.

A potential association between parathyroid function and vitamin D receptor (VDR) polymorphism, via different degrees of VDR mRNA expression in parathyroid tissue depending on VDR subtype (39), has been proposed for 1° hyperparathyroidism (40). In 2° uremic hyperparathyroidism, one group also found such an association (41), but another group did not (40). If an association really exists, a plausible explanation still has to be found regarding how minor variations in noncoding regions of the VDR gene can bring about changes in VDR gene expression.

Role of Phosphate Excess in Vivo

The phosphate ion, which accumulates in advanced stages of renal failure, probably contributes to the development of parathyroid hyperplasia as well. It has long been shown in experimental animal models, as reported by Slatopolsky and Delmez (42), that a phosphate-rich diet induced an increase in parathyroid gland function and volume. More recently, it was found that phosphate-rich diets, when fed to animals with chronic renal failure leading to high plasma phosphate levels, induced parathyroid hyperplasia even when changes in plasma calcium and calcitriol concentration were carefully avoided, pointing to a direct effect of phosphate on cell proliferation (4,16). Conversely, early dietary phosphate restriction was capable of preventing both PTH oversecretion and parathyroid hyperplasia (4,16,28).

Table 2 summarizes the effects of dietary calcium, phosphate, and vitamin D deficiency or excess on parathyroid cell proliferation. However, it is not known at present whether their effects on parathyroid cell growth are direct or indirect. The main reason for this lack of knowledge is that no suitable in vitro models have been available for in-depth studies of the mode of action of these or other factors.

Problem of Parathyroid Cell Culture Models

Present cell culture systems do not generally permit one to maintain functionally active parathyroid cells for prolonged time periods. Such culture systems are characterized by a rapid and significant loss of PTH secretion within 3 to 4 d (43–45). One culture model has been described, using bovine parathyroid cell organoids that maintained the ability to modulate PTH secretion in response to extracellular Ca2+ ([Ca2+]o) and tissue-like morphology for 2 wk in culture (46). However, only one long-term study of bovine parathyroid cells demonstrated a release of bioactive bovine PTH but with reduced sensitivity to calcium (47). It has been demonstrated by others that the rapid decrease in responsiveness of cultured bovine parathyroid cells to changes in [Ca2+]o was associated with a marked reduction in the expression of Ca2+-sensing receptor (Ca-R) (48,49). Other culture models that have been used were parathyroid-derived cells with no PTH secretory capacity (34,50).

An at least partial conservation of the integrity of parathyroid tissue may be a prerequisite for the obtainability of an in vitro response to clinically relevant stimuli. The PTH secretory responsiveness to changes in extracellular phosphate concentration has been shown to depend dramatically on parathyroid cell-to-cell contact, in contrast to the response to [Ca2+]o. Thus, no response at all to an increase in extracellular phosphate was observed with the usual model of dispersed parathyroid cells of bovine or rat origin, whereas a marked response could be obtained when using bovine parathyroid slices (51) or entire rat parathyroid glands (3), respectively.

Despite the limitations of available culture models with respect to long-term conservation of parathyroid cell phenotype, experimental models in vivo have been used to explore the role of diet in mediating parathyroid cell hyperplasia. Several studies have compared the effects of dietary calcium, phosphate, and vitamin D deficiency or excess on parathyroid cell proliferation or hypertrophy.

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type, several groups have attempted to characterize possibly direct effects of calcium and calcitriol, respectively, in vitro. No studies on direct effects of phosphate on parathyroid cell proliferation have been published thus far.

**In Vitro Effect of Calcium**

Surprisingly, at least when considering the issue of parathyroid hyperplasia and the supposed inhibitory effect of calcium thereon in vivo, $[\text{Ca}^{2+}]_e$ generally has not been found to inhibit cell proliferation in vitro, but to enhance it. Such a direct stimulatory effect of $[\text{Ca}^{2+}]_e$ has been demonstrated in various cell types maintained in long-term culture, including fibroblasts, endothelial cells, epithelial cells, and osteoblasts (50,52,53). In intestinal epithelial Caco-2 cells, $[\text{Ca}^{2+}]_e$ appeared to exert this action via the induction of a proto-oncogene (53), and in osteoblastic cells via insulin-like growth factor-I (52). Another recent study showed a weak stimulatory effect of $[\text{Ca}^{2+}]_e$ on DNA synthesis by CCL39 hamster fibroblasts, in which the recombinant rat Ca-R had been expressed. The $[\text{Ca}^{2+}]_e$ effect could be markedly potentiated by concomitant treatment with calcium receptor agonist NPS R-568 (54). Thus, the Ca-R can mediate cell proliferation brought about by $[\text{Ca}^{2+}]_e$.

Increases of $[\text{Ca}^{2+}]_e$ have led to variable results in the model of dispersed bovine parathyroid cells, from an enhancement of cell proliferation (45) to a biphasic response, *i.e.*, first a stimulation when increasing $[\text{Ca}^{2+}]_e$ from low to normal concentrations, and thereafter an inhibition at concentrations above normal (47), or no effect at all (55,56). Ishimi *et al.* failed to observe an effect of $[\text{Ca}^{2+}]_e$ on parathyroid cell number and suggested, based on their experiments, that hyperplasia probably was not caused directly by low $[\text{Ca}^{2+}]_e$ (56). The inhibitory effect of $[\text{Ca}^{2+}]_e$ on cell proliferation noted by Sakaguchi (34) was observed in a cell line derived from rat parathyroid tissue, which turned out to secrete PTHrp, not PTH.

To study direct effects of $[\text{Ca}^{2+}]_e$ on the parathyroid cell in *vitro*, we have recently developed a functional human parathyroid cell culture system capable of maintaining regulation of its secretory activity and the expression of extracellular Ca-R mRNA and protein for several weeks (57). For this purpose, we used parathyroid cells derived from hyperplastic parathyroid tissue of patients with severe 2° uremic hyperparathyroidism. This choice was made because hyperplastic parathyroid cells grew better than cells derived from normal parathyroid glands. The achievement of relatively stable, albeit low degree, cellular proliferation might explain the observed conservation of Ca-R expression, in contrast to previous culture models.

This culture system allows one for the first time to examine the modulation of parathyroid cell growth in *vitro* in a model that remains responsive to $[\text{Ca}^{2+}]_e$ and to study in particular factors directly involved in parathyroid cell proliferation and apoptosis. Preliminary evidence obtained with this model has shown that parathyroid cell proliferation index, as estimated by $[^{3}H]$-thymidine incorporation into an acid-precipitable fraction as a measure of DNA synthesis, could be directly stimulated by high $[\text{Ca}^{2+}]_e$ in the incubation medium (58). It must be pointed out that the response has been variable from one culture experiment to another, corresponding to parathyroid tissues from different glands and/or patients. Varying degrees of response probably reflect underlying pathologic and functional heterogeneity of cells derived from different glands of uremic patients with severe 2° hyperparathyroidism. Moreover, the medium calcium concentration required to induce cell proliferation was often higher than the calcium concentrations observed in the serum of uremic patients.

Our preliminary study showing a direct enhancing $[\text{Ca}^{2+}]_e$ effect has been criticized because parathyroid cells in *vitro* obviously grow much more rapidly than their counterparts in parathyroid tissue in *vivo* (24). Clearly, endocrine cells maintained in culture change from a mainly quiescent to a proliferative phenotype. Such a change applies to most cell culture systems. However, it is only the use of such models that allows one to distinguish indirect from direct effects of compounds involved in parathyroid tissue hyperplasia. Interestingly, our observations are in line with the findings made by Ishimi *et al.*, which were incompatible with a direct effect of low $[\text{Ca}^{2+}]_e$ in the genesis of parathyroid hyperplasia (56). Nonetheless, the extrapolation from *in vitro* observations to the situation in *vivo* should only be done with caution. A possible explanation of the discrepant *in vivo* and *in vitro* findings could be that the stimulatory effects of calcium on cell proliferation *in vitro* are nonspecific for any type, including parathyroid cells devoid of their normal cell contact and growing much more rapidly than they usually do, but that the specific effect of calcium on parathyroid tissue *in situ* is to suppress its proliferation.

**In Vitro Effect of Vitamin D**

Calcitriol has long been shown to inhibit cell proliferation and to enhance cell maturation. Such effects have initially been described in lymphoid cells, and more recently in keratinocytes and myocytes (59–61). Liu *et al.* studied the calcitriol-induced differentiation of the myelo-monocytic cell line U937 by differential screening of a U937 cDNA library. They showed that calcitriol led to the differential expression of the cyclin kinase inhibitor p21$^{WAF1,CIP1}$ (62). They further demonstrated that the p21$^\text{p21}$ gene was transcriptionally activated by calcitriol in a VDR-dependent, but p53-independent, manner and defined a functional vitamin D-responsive element within the p21 promoter mediating this induction. This finding indicates a possible mechanism by which calcitriol could regulate the proliferation of other cells, including parathyroid cells.

In primary cultures of bovine parathyroid cells maintained in short-term culture, Nygren *et al.* (63) showed that these cells underwent significant increases both in number and size in response to fetal calf serum, and that the addition of 10 to 100 ng/ml calcitriol almost completely inhibited cell proliferation, whereas hypertrophy was unaffected. Kremer *et al.* (55) subsequently confirmed in the same parathyroid cell model that calcitriol exerted an antiproliferative action *in vitro*. They further suggested that this inhibition occurred via a reduction of c-myc mRNA expression. There is only one previous report under long-term culture conditions (up to five passages) of the effect of calcitriol on parathyroid cell proliferation, also showing an inhibitory action (56).
In our human parathyroid cell culture model, we examined a possible direct effect of calcitriol as well. Preliminary studies showed that cell proliferation index could be inhibited by high concentrations of calcitriol in the incubation medium (58).

**In Vitro Effect of Phosphate**

There have been no reports thus far, to our knowledge, of a possible direct effect of phosphate on parathyroid cell proliferation. In contrast to calcium and calcitriol, not much attention has been devoted by the scientific community to the role of phosphate on cell growth in general. After extensive literature research into this issue, we have been able to identify only one report dealing with this question (64). The authors examined the effect of increasing phosphate concentrations in the incubation medium on UMR-106 osteoblast-like cells and found an increase in the number of cells and in proliferation index at high phosphate concentration (2 mM), compared with the absence of phosphate.

Taken together, these studies indicate that the involvement of calcium deficiency in the increase of parathyroid tissue mass is complex. Whereas *in vivo* studies suggest an effect stimulating cell hypertrophy and possibly also hyperplasia, at least in conjunction with vitamin D deficiency, results of recent *in vitro* studies are in favor of the hypothesis that the calcium deficiency effect *in vivo* may be indirect, since calcium stimulates cell proliferation *in vitro*. In contrast to the effects of calcium, the suppressive effects of calcitriol administration on the development of parathyroid hyperplasia *in vivo* are in line with the hormone’s inhibitory effects on parathyroid cell proliferation *in vitro*. Whether the observed stimulatory effect of phosphate on parathyroid hyperplasia *in vivo* corresponds to a direct effect remains to be seen in appropriate *in vitro* experiments.

**Nodular Type, Clonal Parathyroid Growth**

In advanced stages of chronic renal failure, changes in the mode of cell growth occur, especially in patients maintained on long-term dialysis treatment. The frequent occurrence of nodular formations within diffusely hyperplastic tissue in parathyroid glands removed from uremic patients with severe 2° hyperparathyroidism, as observed by our group many years ago, was a first hint toward abnormal growth patterns (65). In accord with these findings, in a more recent analysis about 50% of glands weighing between 0.25 and 0.5 g, and more than 90% of glands weighing more than 0.5 g, exhibited nodular formations (66). Such nodules are made of cells more closely packed together than in purely diffuse hyperplasia, with larger nuclei and a greater prevalence of cell cycle markers (67,68), more cycling cells by flow cytometry (69), lower numbers of VDR (6) and calcium-sensing receptors (9,10), and a higher calcium set point for PTH secretion (70). Immunohistochemical analyses of nodular formations revealed similarity in gene expression in cells within the same nodule, but differences between nodules (71). However, detailed morphologic examination including electron microscopy frequently showed heterogeneous aspects of cellular and nuclear size and mitotic features not seen in purely hyperplastic tissue, in glands removed from the neck as well as in parathyroid tissue fragments removed from the forearm following parathyroid autotransplantation (72). Two groups observed an enrichment in oxyphilic cells in nodular areas with higher proliferative activity than in diffusely hyperplastic areas (73,74).

Nodular hyperplasia was reported to be more rapidly progressive and to be characterized by more marked proliferative activity than purely diffuse hyperplasia (Figure 2) (15) (75,76). Proliferative figures were higher in parathyroid glands from dialysis patients with recurrent hyperparathyroidism after neck surgery than from those who remained relatively well controlled (77). Nodular hyperplasia was also associated with a greater resistance to medical suppression of PTH oversecretion (70,75), and recurrence rates of hyperparathyroidism after parathyroidectomy were significantly higher when nodular tissue instead of purely hyperplastic tissue was autografted (78).

More recently, we (79) and others (80,81) have shown, using X chromosome inactivation analysis, that benign monoclonal tumors develop in a high proportion of hyperplastic parathyroid glands, probably favored by the existence of polyclonal parathyroid hyperplasia for many years (23). Multiclonal tumors may also form in some instances (24). We found no correlation between the presence of macroscopically or microscopically evident nodules and the clonal character of resected parathyroid tissue (79), but this may have been due to frequent admixture of polyclonally growing tissue. Several glands with a diffuse hyperplastic appearance also were unequivocally monoclonal in the absence of detectable nodular formations. We assume that monoclonal uremic parathyroid glands, which exhibit a pure hyperplastic appearance and no nodules, correspond to end-stage “nodular” transformation (Figure 3).

The reason for the high frequency of clonal proliferation is unclear. Probably the long-standing stimulation of a tissue with a usually extremely slow growth pattern favors the emergence of more actively proliferating cell clones, as has also been observed in nonuremic patients with multiple endocrine neoplasia type-1 (82). Defective DNA repair mechanisms may play a role as well (83).

![Figure 2. Comparison of PCNA labeling index (LI, per 1000 cells) in secondary hyperparathyroidism of chronic hemodialysis patients, classified by glandular structure. Both the maximum LI (□) and the average LI (■) were significantly higher for the nodular type (15).](image-url)
Several recent studies hint of the probable involvement of mutations or deletions of tumor suppressor genes, and in some instances also of an activation of tumor enhancer genes, using the microsatellite marker technique (81,84). Unexpectedly, allelic loss was observed at numerous chromosome sites, including the X chromosome. The method of comparative genomic hybridization was used to clarify this issue further (85). Chromosomal losses as well as gains were found in 26% of glands, whereas no anomaly could be detected with this technique in 74% of glands. Losses on chromosome 11, the location of the *menin* gene, were found in a small number, i.e., in 3 of 38 (8%) instances, in keeping with a previous observation by Falchetti et al. (86).

We used the candidate gene approach to define possible genetic changes. When screening genomic DNA using Southern blot from 33 patients with severe 2° uremic hyperparathyroidism for possible rearrangement or allelic loss of several gene markers near the *PTH* and the *menin* gene, we found allelic loss of the *Ha-ras* gene in one of 11 (9%) of them (87). Moreover, one in eight uremic patients showed allelic loss of the tumor suppressor gene *WT1*. No rearrangement or allelic loss was detected for *PRAD-1/cyclin 1*, *IGF-2*, *PTH*, or *RET* genes.

The search for mutations of somatic genes involved in the metabolism of calcium, phosphate and vitamin D has remained unsuccessful in parathyroid tissue hyperplasia of uremic patients. Mutations or losses of heterozygosity of the *calcium-sensing receptor* gene (81,88) and the *VDR* gene (81) could not be identified thus far.

Although the frequency of genetic changes in 2° uremic hyperparathyroidism has been found to be lower than in primary adenoma and carcinoma, the occurrence of such alterations indicates that acquired, adverse genetic events are involved in the pathogenesis of clonal cell proliferation in advanced forms of the 2° hyperparathyroidism of dialysis patients.

Whether benign parathyroid tumors may evolve into malignant forms is still subject to debate. Because parathyroid carcinoma is a rare event in dialysis patients (89–91), malignant transformation of clonal parathyroid neoplasms must be exceptional. However, it appears to be more common than would be expected by chance. Thus, according to Miki et al. (89), 4% of all cases of parathyroid cancer have been observed in dialysis patients.

**Apoptosis of Parathyroid Tissue in Chronic Renal Failure**

Whether a change in the rate of apoptosis also contributes to parathyroid tissue hyperplasia is still a matter of debate (21,24,25). One research group recently examined this issue in rats with short-term renal failure (5 d) and failed to detect apoptosis in their hyperplastic parathyroid glands (17). The explanation for this failure could be lack of sensitivity of the employed methods, as discussed above.

The constantly negative finding in rats contrasts with observations of significant apoptotic figures in hyperplastic parathyroid tissue of humans. Wang et al. (19) found evidence of apoptosis in 85% of primary (1°) adenomas. Three of the cases examined harbored more than 5% apoptotic cells. Apoptosis was also found in 13 of 15 remnants of normal tissue outside the adenoma capsule, as well as in two normal parathyroid glands removed from patients who underwent neck surgery for parathyroid-unrelated disease.

These findings are similar to observations initially made by our group (20), with 1.3, 5.3, and 9.5% apoptotic cells, respectively, in normal parathyroid tissue, 1° parathyroid adenoma, and 2° uremic parathyroid hyperplasia. However, we found that in hyperplastic tissue TUNEL-positive cells frequently were not evenly distributed but exhibited focal accumulation.

The numbers (percentage) of apoptotic cells found by Wang et al. (19) and in our initial study were approximately 10-fold higher than those reported by two other groups (18,92). Therefore, we examined apoptosis in a new series of parathyroid tissue sections, using a technically refined, highly reproducible TUNEL method. In this work, we again found roughly 10-fold higher apoptotic figures in tissues from uremic patients than in normal parathyroid tissue (Figure 1) (22). Absolute values,
however, were lower by one order of magnitude than in our earlier study (20). It should be noted again that although the frequency of apoptosis is higher in uremic than in normal parathyroid glands, overall it is very low, as expected for low turnover endocrine tissues in general (24).

Interestingly, the uremic state appears to stimulate apoptosis in other cell types such as the circulating monocyte (93), possibly via the well known increase of cytosolic Ca^{2+}, which has been observed in a variety of cells in renal failure (94), and also via the noxious effect of nonbiocompatible dialysis membranes used for renal replacement therapy (95).

If confirmed by others, the observed enhancement of parathyroid tissue apoptosis could compensate, at least in part, for the increase in parathyroid cell proliferation observed in 2° uremic hyperparathyroidism.

**Regression of Parathyroid Hyperplasia**

Theoretically, increases in parathyroid gland mass due to endogenous or exogenous stimuli should be reversible in case such stimuli cease to exist. However, whether regression of parathyroid hyperplasia actually occurs in animals or patients with chronic renal failure remains controversial. According to some authors, regression must be an extremely slow process, if it occurs at all (16,24). This is in sharp contrast to the rapid reversibility of excessive parathyroid function in uremic rats after normalization of renal function by kidney transplantation (96), although parathyroid mass probably remained markedly increased in this experimental model.

The issue of regression is of clinical importance. If, for example, a chronic dialysis patient with a dramatic increase in total parathyroid mass to 5000 mg (from a normal total mass of 200 mg) has practically no chance to see his hyperplasia regress toward normal after uremia correction by a successful kidney graft, it would seem appropriate to perform a surgical parathyroidectomy before renal transplantation, instead of exposing the patient to the risk of developing hypercalcemia and associated complications. If, however, significant regression of hyperplasia occurs as an active or passive process, namely by enhanced apoptosis or reduced proliferation, prophylactic surgery should be avoided.

**Evidence from Animal Experiments**

That regression of parathyroid hyperplasia secondary to vitamin D deficiency can occur has been convincingly demonstrated by Henry et al. in the experimental animal as early as 1977 (97). These authors showed that the administration of cholecalciferol to chicks that had developed an increase in parathyroid gland mass when fed a rachitogenic, vitamin D-free diet for 8 to 10 wk led to a significant (50%) reduction in gland weight. Calcitriol failed to achieve the same effect at low, albeit hypercalcemic, doses, but was capable of reducing gland mass at a higher dose. The reduction of gland weight went along with a loss of parathyroid cells, indicative of regression of hyperplasia.

In dogs submitted to a low calcium, low sodium, and vitamin D-deficient diet for 2 yr, the effect of the subsequent reintroduction of normal diet on parathyroid function was studied in a detailed manner, including the determination of intact and C-terminal PTH (98). Calculations of PTH secretion in response to variations in plasma calcium allowed the authors to deduce that there was no involution of hyperplastic parathyroid glands despite the return to normal diet for 17 mo.

In uremic animals, published evidence for or against regression of increased parathyroid mass is sparse. The calcimimetic drug NPS R-568, which has been shown to prevent parathyroid hyperplasia in 5/6 nephrectomized rats, was unable to reverse it (17).

**Evidence in Patients**

Rapid remission of parathyroid overfunction may occur either due to parathyroid "apoplexy," i.e., necrosis, as has been shown in rare cases of 1° hyperparathyroidism (99), or due to enhanced apoptosis (Figure 4). The diagnosis of necrosis is more difficult in 2° than in 1° hyperparathyroidism because most often the hyperplasia of the former is not limited to one gland.

Regression of parathyroid hyperplasia in hemodialysis patients in response to calcitriol pulse therapy for 12 wk has been reported by Fukagawa et al., using ultrasonography (100). These authors observed a significant decrease in mean gland volume from 0.87 to 0.51 cm^3 during this time period, concomitantly with a reduction in serum iPTH of more than 50%. In contrast, Quarles et al., who also examined parathyroid gland anatomy in hemodialysis patients in vivo in response to intermittent intravenous or oral calcitriol treatment for 36 wk, failed to observe a decrease in parathyroid gland size as assessed by high resolution ultrasound and/or magnetic resonance imaging (101). Mean gland size was 1.9 and 2.1 cm^3 before and 3.3 and 2.3 cm^3 after oral and intravenous calcitriol treatment, respectively. The authors achieved an overall maximum average serum PTH reduction of 43% over this time period.

Note that there were important differences between these two studies. Hyperparathyroidism probably was more severe in the latter. Although initial mean serum iPTH levels were similar, serum phosphorus was higher and the decrease in

**Figure 4.** Two theoretical pathways of regression of parathyroid hyperplasia.
serum PTH achieved in response to calcitriol was less marked in the latter. Moreover, parathyroid mass was more than double.

In another study, Fukagawa et al. examined the possible relation between parathyroid size and the long-term outcome after calcitriol pulse therapy, by subdividing patients into different groups according to estimation of initial parathyroid gland volume (102). In two hemodialysis patients with detectable gland(s), in whom the size of all parathyroid glands as well as PTH hypersecretion regressed to normal, 2° hyperparathyroidism remained controllable for at least 12 mo after switching to conventional oral active vitamin D therapy. In contrast, in seven hemodialysis patients, in whom the size of all parathyroid glands did not regress to normal by calcitriol pulse therapy, 2° hyperparathyroidism relapsed after switching to conventional therapy, although PTH hypersecretion could be controlled temporarily.

Taken together, these findings suggest that the degree of parathyroid hyperplasia, as detected by ultrasonography, may be an important determinant for regression in response to calcitriol therapy. It is probable, although not proven, that the type of hyperplasia, i.e., monoclonal, multiclonal, or polyclonal growth, is more important with respect to regression than the actual size of each gland. Another, at least theoretical, possibility is that reduction in parathyroid gland size occurs due to reversal of hypertrophy and reduction of hypervascularity, in case the latter two are present in addition to hypercellularity, and depending on the inducibility of apoptosis, which may be variable from one gland to the other.

The mechanisms involved in the hyperplasia of parathyroid glands in 2° uremic hyperparathyroidism are less well understood than the mechanisms governing excessive PTH synthesis and release. The major reason for this has been the lack of appropriate cell culture models for the study of parathyroid cell proliferation and apoptosis. In chronic renal failure, the increase in parathyroid mass is mainly due to enhanced cell proliferation, not cell hypertrophy. Schematically, one can distinguish a first stage of diffuse, polyclonal hyperplasia from a second stage of often nodular monoclonal or multiclonal hyperplasia.

The main factors responsible for excessive polyclonal parathyroid tissue growth appear to be the same as those involved in excessive PTH synthesis and secretion, i.e., calcium, phosphate, and vitamin D. In addition, a decrease in calcium and vitamin D sensing by their respective receptors in the parathyroid cell, as well as an increase in the expression of growth factors and a reduction in the expression of growth inhibitory factors, could also play a role.

The switch to clonal growth probably involves various acquired mutations or allelic losses of tumor enhancer and tumor suppressor genes. The occurrence of such mutations or losses may be favored by the long-standing stimulation of this normally slow-growing endocrine tissue and also by defective DNA repair mechanisms in chronic renal failure.

According to our findings, the increase in parathyroid cell proliferation is associated with an accelerated rate of apoptosis. However, because parathyroid gland mass progressively grows with time in many uremic patients on long-term dialysis treatment, the increase in apoptosis is insufficient to counterbalance enhanced proliferation in the long run. Whether an inversion of this imbalance, i.e., regression of parathyroid hyperplasia, can ever occur in uremic patients remains a matter of debate.

References

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