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1α,25-Dihydroxycholecalciferol Reduces Rejection and Improves Survival in Rat Liver Allografts

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Vitamin D_3 affects the immuno response and improves experimental autoimmune diseases. We investigated the effect of 1,25-dihydroxycholecalciferol ($1,25[OH]_2D_3$) Rocaltrol as a single immunosuppresive agent and in combination with low-dose cyclosporin A (CsA) in vascularized liver allografts in rats in a high-responder strain combination (ACI⇒Lewis). Recipients were placed on a low-calcium diet 7 days before transplantation and were treated with 0.1 or 1 μg/kg/d $1,25(OH)_2D_3$ intraperitoneally beginning 3 days before transplantation. Treatment combining $1,25(OH)_2D_3$ with CsA (2 mg/kg/d) was also tested. Graft function and survival, histologic rejection, and concentrations of interleukin (IL)-2, -4, -10, and -12 in serum and in grafts were measured. $1,25(OH)_2D_3$ increased allograft survival in a dose-dependent manner when compared with controls ($P < .05$ for both groups). Serum bilirubin, aspartate transaminase (AST), and lactate dehydrogenase (LDH) activities were significantly lower in $1,25(OH)_2D_3$-treated animals. Vitamin D reduced the concentration of IL-2 and IL-12 in serum and in grafts, and increased IL-4 and IL-10 in the grafts. The rejection activity index 10 days after transplantation was significantly lower in low- and high-dose $1,25(OH)_2D_3$-treated rats compared with vehicle-treated controls ($P < .0001$ for both groups). The combination of either low-dose or high-dose vitamin D_3 and CsA prolonged graft survival when compared with low-dose CsA only ($P < .05$ for both groups). After 3 weeks, hypercalcemia developed in high-dose $1,25(OH)_2D_3$-treated rats. It is concluded that $1,25(OH)_2D_3$ prolongs survival of liver allografts in rats by decreasing the severity of acute rejection. Analogues of vitamin D with fewer hypercalcemic effects may have potential as immunosuppressive drugs in liver transplantation. (HEPATOLOGY 2001;34:926-934.)

The active form of vitamin D, $1,25(OH)_2D_3$, is a physiologically occurring molecule with immunomodulatory effects, and it has previously been shown that activated T lymphocytes, macrophages, and monocytes express the vitamin D receptor.[1-4] Upon binding to its specific receptor, $1,25(OH)_2D_3$ exerts a variety of immunomodulatory effects such as inhibition of T-lymphocyte proliferation,[5-7] downregulation of cytokine production such as interleukin 2 (IL-2) and interferon gamma,[6,8-15] and suppression of Th1-helper lymphocytes *in vitro* and *in vivo*.[16,17] Vitamin D has been found to inhibit in a time- and dose-dependent fashion the generation of cytotoxic natural-killer cells, and this effect can be attenuated by administration of exogenous IL-2.[10,18] In addition, recent data have shown that vitamin D reduces IL-12 expression in macrophages and dendritic cells.[19-21] In contrast, $1,25(OH)_2D_3$ promotes the expression of the Th2 cytokines, IL-4 and IL-10.[22,23]

These immunomodulatory effects of vitamin D and its analogues have been examined in several experimental models of autoimmune diseases such as encephalomyelitis,[17,24] thyroiditis,[25] arthritis,[26] and nephritis.[27] These results suggest that $1,25(OH)_2D_3$ may have therapeutic potential in other clinical settings in which inhibition of Th1-helper lymphocyte function is mandatory, such as allotransplantation. Indeed, it was reported that $1,25(OH)_2D_3$ prolongs survival of cardiac allografts *in vivo*.[28] As a result of the hypercalcemic effects, vitamin D has not been used in clinical practice so far. However, it was recently shown that vitamin D derivatives might potentiate the effect of calcineurin inhibition *in vitro* and *in vivo*.[29-31]

The effects of vitamin D have never been studied in liver transplantation. Therefore, we investigated whether the administration of $1,25(OH)_2D_3$ affects liver allograft viability and rejection in a rat animal model. Furthermore, expression of IL-2 and IL-12, as well as IL-4 and IL-10, were determined to examine the immunomodulatory effects of $1,25(OH)_2D_3$. In a second set of experiments, the combined effect of $1,25(OH)_2D_3$ together with cyclosporin A (CsA) on graft survival and cytokine expression was determined.

MATERIALS AND METHODS

Animals and Experimental Design

Animal experiments were performed in accordance with the national guidelines for the care and use of laboratory animals and followed approval by the local authorities. Male inbred Lewis (RT1.l) and ACI (Rt1.a) rats (300-350 g), obtained from Harlan Sprague Dawley (Horst, the Netherlands), were used in a high-responder strain combination (ACI⇒Lewis). Recipient rats were placed on an experimental diet low in calcium and devoid of vitamin D, as previously described,[24] 7 days before and following transplantation. Tap

Abbreviations: $1,25(OH)_2D_3$, 1α,25-dihydroxycholecalciferol; IL, interleukin; CsA, cyclosporin A; AST, aspartate transaminase; LDH, lactate dehydrogenase; ELISA, enzyme-linked immunosorbent assay; PBS-T, phosphate-buffered saline with 0.05% Tween; OD, optical density.

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