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Cecilia Johnsson
Gunnar Tufveson

MC 1288 – A vitamin D analogue with immunosuppressive effects on heart and small bowel grafts

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G. Tufveson (✉)
Department of Transplantation Surgery,
University Hospital,
S-75185 Uppsala, Sweden
Fax: +46 18 553 601

C. Johnsson
Departments of Transplantation Surgery and Pediatric Surgery, University Hospital,
S-751 85 Uppsala, Sweden

Abstract The vitamin D analogue MC 1288 (20-epi-1α,25-dihydroxycholecalciferol) was tested here for its possible immunosuppressive properties in vivo using different rat transplantation models. MC 1288, in a dose of 0.1 μg/kg daily, administered intraperitoneally for 10 days, was found to be effective in prolonging cardiac allograft survival. Untreated recipients rejected their grafts around day 8 while MC 1288 treatment delayed rejection until day 22 ($P < 0.001$). Addition of the immunostimulatory drug LS-2616 (Linomide) reduced the immunosuppressive effect of MC 1288 and rejection occurred around day 11. The immunosuppressive effect of MC 1288 on rejection following small bowel transplantation was determined by measuring the amounts of hyaluronan (HA) secreted into the intestinal lumen. On day 6 post-transplantation the amounts of intraluminal HA in untreated animals was 29.2 ± 5.3 ng/min and cm, while in MC 1288-treated animals it was just 5.0 ± 1.6 ng/min and cm ($P < 0.01$). We conclude that MC 1288 has immunosuppressive effects that may make it suitable for the prevention of graft rejection.

Key words Vitamin D analogues, immunosuppression · Immunosuppression, vitamin D analogues · Heart transplantation, vitamin D analogues · Small bowel transplantation, vitamin D analogues

Introduction

1α,25-dihydroxycholecalciferol [1α,25$(OH)_2D_3$], the active form of vitamin D, has been shown to possess immunoregulatory properties. Receptors for 1α,25$(OH)_2D_3$ are expressed on monocytes and on activated T and B cells [2, 15]. In vitro the substance inhibits the T-cell production of interleukin-2 (IL-2) [12, 17], interferon-gamma (IFN-γ) [16, 19], and granulocyte-macrophage colony-stimulating factor (GM-CSF) [23], and antigen- or mitogen-activated T cells incubated with 1α,25$(OH)_2D_3$ show a decreased proliferation rate [17]. Because of the strong effects exerted on calcium metabolism, systemic use of 1α,25$(OH)_2D_3$ for treatment of immunological disorders is limited, as it may lead to the development of hypercalcemia and hypercalciuria [25]. For this reason, new vitamin D analogues are being synthetized and investigated with regard to their potency on the immune system. One such analogue is MC 1288 (20-epi-1α,25-dihydroxycholecalciferol), which only differs from 1α,25$(OH)_2D_3$ in the altered stereochemistry at carbon 20 (Fig. 1). MC 1288 has much stronger effects than 1α,25$(OH)_2D_3$ on T-cell activation in vitro. In a mouse thymocyte costimulatory assay, the substance was over 7000 times more potent than 1α,25$(OH)_2D_3$, while in inhibiting allogeneic T-cell activation, as investigated in a mixed lymphocyte reaction, it was nearly 300 times more potent [3].

An important aspect of the development of new immunosuppressive drugs is their use in organ transplantation. Here, we have evaluated the immunosuppressive effects of MC 1288 in various rat transplantation mod-

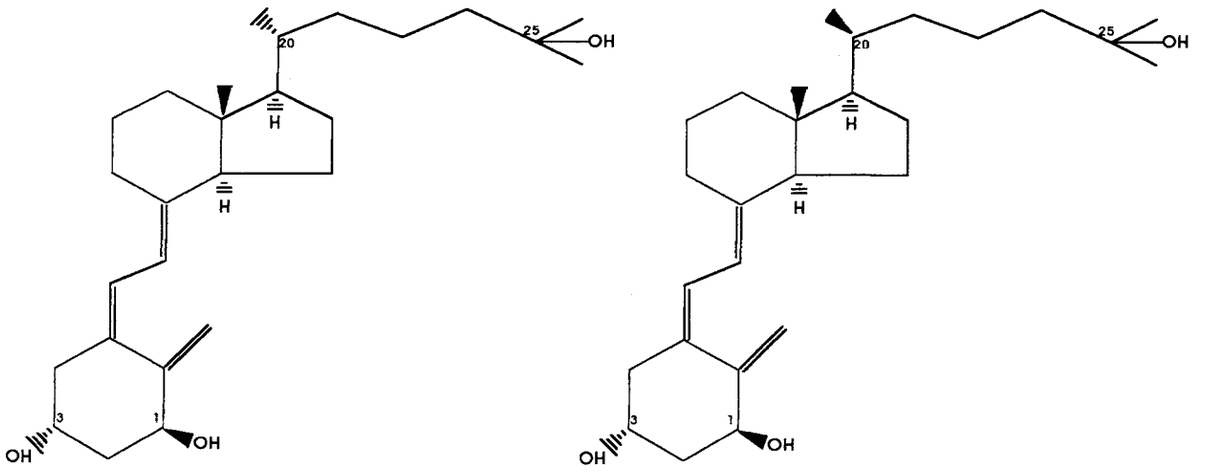

Fig. 1 Molecular structure of 1α,25-dihydroxycholecalciferol [1α, $25(OH)_2D_3$] and 20-epi-1α,25-dihydroxycholecalciferol (MC1288). The only difference between the two molecules is the stereochemistry at carbon 20

els, one being the traditional heterotopic cardiac allograft model, which is excellent in screening for potential immunosuppressants. In order to enhance this model, immunosuppressive treatment can be combined with an immunostimulator. The immunostimulating agent LS-2616 (Linomide), which totally abrogates the immunosuppressive effect of cyclosporin A (CyA) [27], was used here for this very purpose. MC 1288 was also tested in the more complicated heterotopic small bowel transplantation model, where rejection is more difficult to control than after heart transplantation. Since it is rather difficult to define the day of rejection after small bowel transplantation, we have used a technique for the determination of hyaluronan (hyaluronic acid; HA) secreted into the intestinal lumen, something which has previously been demonstrated to be a sensitive marker for rejection [7]. Finally, the effects of MC 1288 were tested on graft-versus-host reaction (GVHR) after small bowel transplantation. This immunological event is even harder to control by immunosuppressive agents than is rejection.

Materials and methods

Animals

Male PVG, Wistar/Kyoto (Wi/Ky), Lewis, and (LewisxBN)F_1-hybrid (LxBN) rats were obtained from Møllegaard (Skensved, Denmark). The animals were allowed to get settled for at least 1 week before the experiments were started and had, during the whole period, free access to food and water. At the time of operation, rats that were to undergo heart transplantation weighed 150–250 g and those to undergo small bowel transplantation weighed 210–270 g. Heart recipients and small bowel donors and recipients were anesthetized with a mixture of chloral hydrate (180 mg/kg body weight), pentobarbital (40 mg/kg body weight), and magnesium sulphate (90 mg/kg body weight), administered intraperitoneally. Heart donors were anesthetized by intraperitoneal injections of Inactin (Byk, Gulden, Konstanz, Germany), 120 mg/kg body weight. The experiments were approved of by the regional ethical committee and treatment of the animals conformed to the "Principles of laboratory animal care."

Immunomodulating drugs

MC 1288 (20-epi-1α,25-dihydroxyvitamin D_3) was a kind gift from Dr. L. Binderup (Leo Pharmaceutical, Ballerup, Denmark). The drug was dissolved in propylene glycol (Riedel-de Haën, Seelze, Germany) and a volume of about 100 µl was injected intraperitoneally daily for 10 days, starting on the day of transplantation.

LS-2616 (Linomide; Kabi Pharmacia, Helsingborg, Sweden) was dissolved in tap water and administered orally via a gastric feeding tube. A total of 160 mg/kg body weight was given daily, starting on day – 1, for as long as the grafts continued to function.

Experimental groups

The animals were assigned to nine different experimental groups, as indicated in Table 1.

Heart transplantation

Hearts from PVG donors were transplanted heterotopically into Wi/Ky recipients. The aorta of the donor heart was anastomosed to the common carotid artery of the recipient and the pulmonary artery to the jugular vein using a nonsuture technique [14]. In brief, the carotid artery and the jugular vein were dissected free, crossclamped caudally, and cut cranially. Short plastic tubes were placed around the vessels, and the vessels were then turned inside out over the tubes and fixed with ligatures. The heart was anasto-

Table 1 Experimental groups

Heart transplantation, PVG → Wi/Ky	
Group 1 ($n = 8$)	Untreated
Group 2 ($n = 6$)	MC 1288, 0.05 μg/kg
Group 3 ($n = 8$)	MC 1288, 0.1 μg/kg
Group 4 ($n = 11$)	MC 1288, 0.1 μg/kg; LS-2616, 160 mg/kg
Small bowel transplantation, rejection	
Group 5 ($n = 8$)	LxBN → Lewis, untreated
Group 6 ($n = 6$)	LxBN → Lewis, MC 1288, 0.1 μg/kg
Group 7 ($n = 8$)	Lewis → Lewis, untreated
Small bowel transplantation, GVHR	
Group 8 ($n = 9$)	Lewis → LxBN, untreated
Group 9 ($n = 6$)	Lewis → LxBN, MC 1288, 0.1 μg/kg

mosed, pulling the vessels of the graft over the tubes, and the vessels were fastened with ligatures. When the transplantation was finished, a single dose of cefuroxim (Zinacef, Glaxo, Greenford, UK), 20 mg/rat, was given intramuscularly. Graft function was monitored by daily palpation, and rejection was defined as the day when no pulsations were detectable.

Small bowel transplantation

The bowel was dissected free on a vascular pedicle consisting of the superior mesenteric artery and the portal vein. After heparinization (300 IU/rat) the aorta was crossclamped proximally and tied off distally to the superior mesenteric artery. A catheter was inserted and the vascular system perfused with a low pressure (35 cm H_2O) using a cold (4 °C) balanced salt solution containing histidine and mannitol (FW solution) [5]. In the meantime, the lumen was irrigated with 20 ml of the same FW solution. Until transplantation the bowel was stored at 4 °C in FW solution. The bowel was transplanted heterotopically to the left renal vessels of the recipient using the same nonsuture technique as described above [14, 26]. After restoring the blood flow, the proximal end of the bowel was closed blindly and the distal end anastomosed end-to-side to the distal ileum of the recipient's native bowel. A single dose of cefuroxim, 20 mg/rat, was given intramuscularly.

Assessment of GVHR

Animals were weighed and carefully inspected daily for signs of GVHR. GVHR after semisyngeneic small bowel transplantation is characterized by redness of the ears and paws, progressing to dermatitis, secretion from the nose and eyes, hair loss, diarrhea, and loss of weight.

Segmental perfusion

Segmental perfusion of the intestine was performed as described elsewhere [7]. In brief, rats were anesthetized on day 6 and a 4-cm long segment of the distal part of the transplanted bowel was dissected free. Plastic catheters were carefully inserted into each end of the segment and fixed with ligatures. The proximal catheter was connected to a mechanical pump (ASID, Bonz) through which 0.9 % NaCl was infused into the bowel at a flow rate of 0.2 ml/min. Via the distal catheter perfusate was collected in test tubes at 10-min intervals. The collected fluid was centrifuged at 2000 g for 15 min, after which the supernatants were stored at − 70 °C until analyzed.

Quantitative assay of HA

A commercially available radiometric assay (Kabi Pharmacia, Uppsala, Sweden) was used to determine the amounts of HA in each test tube. The technique is based on the binding of HA to specific hyaluronic acid-binding proteins (HABP) [22]. Briefly, a 100-μl sample is incubated for 60 min at 4–7 °C with 200 μl ^{125}I-labelled HABP. Then, 100 μl HA-sepharose is added and the incubation continues for 45 more min at the same temperature. Before centrifugation at 2000 g for 10 min, 2 ml of washing solution is added. After decantation the radioactivity in the pellet is measured in a gamma counter. A standard curve is constructed from samples with known amounts of HA. Double analyses were performed on each sample. The HA values are expressed as amount secreted per minute and centimeter bowel, to correct for the fact that the length of the segments perfused differed somewhat between the animals.

Statistical analysis

Data are expressed as median values and ranges or as mean values and standard error of the means (SEM). Differences between groups were evaluated using the Mann-Whitney U-test (graft survival) or Student's t-test (HA analysis). A P value below 0.05 was considered statistically significant.

Results

Heart transplantation

Untreated Wi/Ky recipients of PVG cardiac allografts rejected their grafts within 9 days and had a median survival time of 8 (range 7–9) days (Fig. 2). Treatment with MC 1288 in a daily dose of 0.1 μg/kg prolonged graft survival to 22 (range 16–27) days ($P < 0.001$ compared to the untreated group). The lower dose, 0.05 μg/kg, produced prolonged graft survival in some animals, while in others no effect could be seen. The median survival time in this group was 12 (range 8–22) days ($P < 0.05$ compared to the untreated group). The addition of LS-2616 resulted in the reversal of the immunosuppressive effect induced by MC 1288 ($P < 0.001$); this effect was seen in 10 out of 11 animals thus treated.

Small bowel transplantation – rejection

Analyses of the amount of HA secreted into the intestinal lumen of the transplanted bowel on day 6 showed a mean value of 5.0 ± 1.6 ng/min and cm during the first 10 min of perfusion in animals treated with MC 1288, while in untreated animals it was 29.2 ± 5.3 ng/min and cm (Fig. 3). In untreated, syngeneically transplanted animals the amount of HA secreted was 2.6 ± 1.0 ng/min and cm.

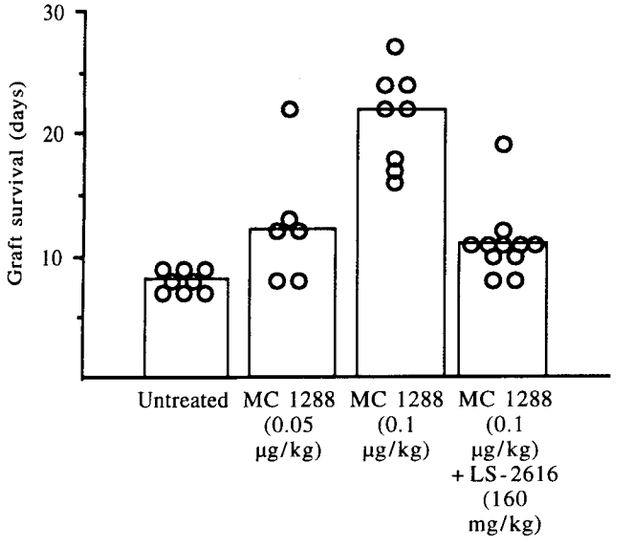

Fig. 2 The effect of treatment with the vitamin D analogue MC 1288 alone or in combination with the immunostimulatory substance LS-2616 on heart allograft survival. MC 1288 was given on days 0–9 in a daily dose of 0.05 or 0.1 μg/kg; LS-2616 was given in a daily dose of 160 mg/kg as long as the grafts were functioning, starting on day – 1. Each circle represents one animal. The height of the bar represents the median graft survival time in each experimental group

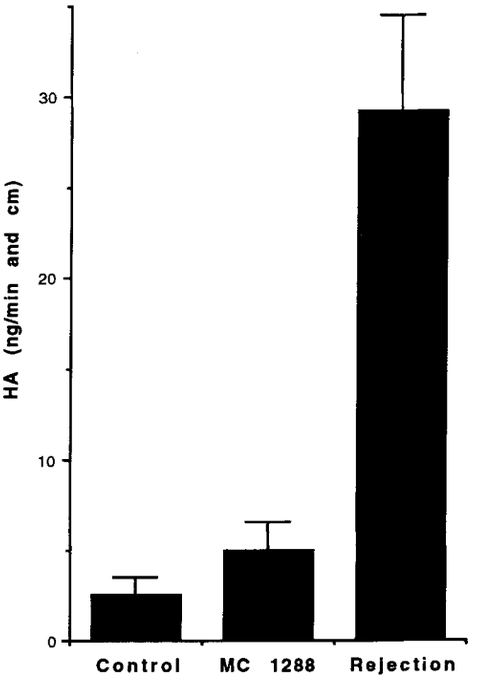

Fig. 3 The recovery of hyaluronan *(HA)* during the first 10 min of perfusion of small bowel grafts following syngeneic transplantation *(control)*, semiallogeneic transplantation with treatment of the recipient with MC 1288, 0.1 μg/kg *(MC 1288)*, or semiallogeneic transplantation with untreated recipients *(rejection)*. Data are expressed as mean values and standard error of the mean

Small bowel transplantation – GVHR

In untreated semisyngeneically transplanted animals, signs of GVHR first appeared around day 7, whereafter the disease progressed until death occurred around day 14 (range 10–16; Fig. 4). Treatment with MC 1288 had no beneficial effect and the median survival time in this group was 9.5 (range 7–14) days. The two animals surviving for 14 days developed visible signs of GVHR somewhat later than normal, while the rest died without any signs of GVHR.

Discussion

In the present study, treatment of heart graft recipients with the vitamin D analogue MC 1288, in a dose of 0.1 μg/kg for 10 days, was shown to significantly prolong graft survival. Median graft survival times for MC 1288-treated animals was 22.0 days, while for untreated animals it was just 8.0 days. The optimal dose in the heart rejection model appeared to be 0.1 μg/kg since half this dose was effective in only some animals and higher doses did not prolong graft survival further (data not shown).

When screening for immunosuppressive substances, it is common to compare effects and mechanisms of action with those of CyA. In inhibiting allogeneic T-cell activation, MC 1288 is about 2.7×10^4 times more potent than CyA [3]. In the cardiac allograft model used here, Wi/Ky recipients of PVG hearts treated with 10 mg/kg CyA on days 0–9 rejected their grafts around day 19 [4, 27], which is at about the same time we observed rejection in animals treated with MC 1288 in a dose of 0.1 μg/kg.

The immunostimulating substance LS-2616 totally abrogates the immunosuppressive effect of CyA [27] and has thus been used to study rejection under what seems to be adequate immunosuppression, a situation not uncommon after human organ transplantation. LS-2616 stimulates natural killer (NK) cells [8] and macrophages [10], and enhances the in vitro production of IL-1, IL-2, tumor necrosis factor-alpha (TNF-α), and IFN-γ [1, 10], but its exact mechanisms of action in transplantation models are not yet ascertained. MC 1288, in combination with LS-2616, gave a median graft survival time of 11.0 days. This finding is more in accordance with the results obtained after low-dose treatment with 15-deoxyspergualin together with LS-2616 than with those after combined CyA and LS-2616 treatment [4], indicating that CyA and MC 1288 do not exert their immunosuppressive effects via exactly the same mechanisms.

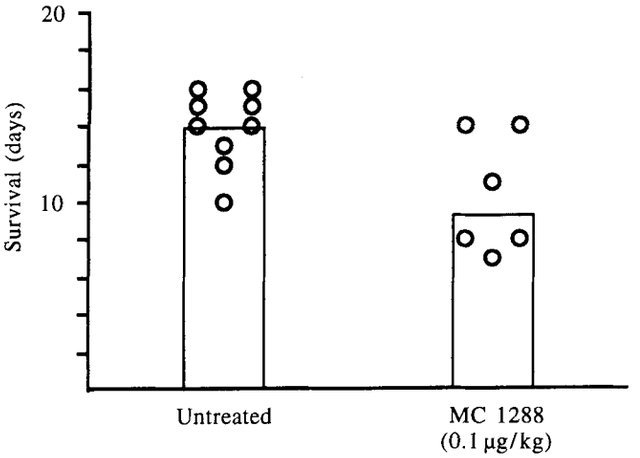

Fig. 4 The effect of treatment with the vitamin D analogue MC 1288 on graft-versus-host reaction after small bowel transplantation. MC 1288 was administered on days 0–9 in a daily dose of 0.1 μg/kg. Each circle represents one animal. The height of the bar represents the median survival time in each experimental group

Rejection after small bowel transplantation appears to be more difficult to control than rejection occurring after transplantation of other organs, and in rat models usually high doses of immunosuppressants have to be used in order to achieve long-term functioning grafts. Also, it is difficult to define the day of rejection after experimental small bowel transplantation by noninvasive methods. The release of HA into the intestinal lumen has been found to correlate well with the course of rejection [7] and can thus be used to measure the efficacy of an immunosuppressive treatment. Using this technique we conclude that MC 1288, in the same dose as in heart transplantation, also has a beneficial effect in preventing rejection of small bowel grafts.

GVHR is often even more difficult to control than rejection, and in the one-way GVHR model we could not observe any positive effect of MC 1288 at all. We can only speculate as to the reasons for this lack of effectiveness. First, the dose may be insufficient, but since not even a slight effect was observed, this does not seem to be a plausible explanation. Second, the mechanisms behind GVHR and rejection may be different [6], resulting in the possibility that MC 1288 may be able to control rejection but not GVHR. Most immunosuppressive drugs investigated can, however, postpone GVHR to some degree.

$1\alpha,25(OH)_2D_3$ has been demonstrated, by several investigators, to affect the production of various cytokines by activated T cells. Inhibition of IL-2 [12, 17], IFN-γ [16, 19], and GM-CSF [23] has been observed. Expression of the IL-2 receptor is, however, not altered [12, 18]. $1\alpha,25(OH)_2D_3$ can also inhibit the production of IL-1 by monocytes [24]. Since IL-1 stimulates T-cell activation, some of the immunoregulatory effects exerted by $1\alpha,25(OH)_2D_3$ may be due to this effect on monocytes [24]. Others have, however, found that the presence of monocytes is not necessary for the inhibition of T-lymphocyte proliferation [9]. The effect on T-helper/inducer lymphocytes appears to be responsible for both the suppression of T-cell proliferation and the inhibition of immunoglobulin production by B cells [12].

Vitamin D analogues with an altered stereochemistry at carbon 20 – and MC 1288 is one example of this – have much more potent effects on cell growth regulation than $1\alpha,25(OH)_2D_3$ itself. The reason for this is not clear, but postreceptor binding effects, such as higher stability of the drug-receptor complex rather than increased affinity for the receptor, appear to be responsible for this increased potency [3]. The calcemic effect of MC 1288 is of about the same order as that of $1\alpha,25(OH)_2D_3$, but since MC 1288 is effective at much lower concentrations, there appears to be no obvious side effect on calcium metabolism. Thus, after 6 days of treatment with MC 1288 in doses of 0.05 or 0.1 μg/kg, just a moderate increase in serum calcium levels was found in nontransplanted animals (data not shown).

Some in vivo experiments have been performed in order to elucidate the immunoregulatory effects of $1\alpha,25(OH)_2D_3$ and its analogues. For instance, prevention of the development of experimental autoimmune encephalomyelitis (EAE) [11] and prolongation of nonvascularized heart grafts [13] have been observed. In patients on hemodialysis, treatment with $1\alpha(OH)D_3$ restores IL-2 production and lymphocyte functions [20, 21]. Similar effects have been seen in experiments using vitamin D-deficient mice [28]. These observations, together with the in vitro findings of stimulatory effects on monocytes/macrophages, indicate that $1\alpha,25(OH)_2D_3$ possesses both immunostimulating and immunosuppressive properties, depending on the physiological state.

Taken together, our results in rat transplantation models indicate that, in the future, vitamin D analogues – preferably the potent 20-epi-vitamin D_3 analogues – may be used as immunosuppressive agents for controlling graft rejection. The analogue MC 1288 has shown promising immunosuppressive effects on both cardiac and small bowel grafts.

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References

1. Bengtsson M, Simonsson B, Carlsson K, Nilsson B, Smedmyr B, Termander B, Öberg G, Tötterman TH (1992) Stimulation of NK cell, T cell, and monocyte function by the novel immunomodulator Linomide after autologous bone marrow transplantation. Transplantation 53: 882–888
2. Bhalla AK, Amento EP, Clemens TL, Holick MF, Krane SM (1983) Specific high-affinity receptors for 1,25-dihydroxyvitamin D_3 in human peripheral blood mononuclear cells presence in monocytes and induction in T lymphocytes following activation. J Clin Endocrinol Metab 57: 1308–1310
3. Binderup L, Latini S, Binderup E, Bretting C, Calverley M, Hansen K (1991) 20-Epi-vitamin D_3 analogues: a novel class of potent regulators of cell growth and immune responses. Biochem Pharmacol 42: 1569–1575
4. Gannedahl G, Wanders A, Carlsson M, Tufveson G (1993) 15-Deoxyspergualin effects in rat heart allograft transplantation – relation to dose, timing, and cyclosporine. Transplantation 55: 455–456
5. Jacobsson J, Wahlberg J, Frödin L, Odlind B, Tufveson G (1989) Organ flush out solutions and cold storage preservation solutions. Effect on organ cooling and post ischemic erythrocyte trapping in kidney grafts. An experimental study in the rat. Scand J Urol Nephrol 23: 219–222
6. Johnsson C, Tufveson G (1994) The immunomodulator LS-2616 (Linomide) more effectively than sensitization of the donor enhances graft-versus-host reaction after small bowel transplantation. Transplant Immunol 2: 56–60
7. Johnsson C, Hällgren R, Tufveson G (1993) Recovery of hyaluronan during perfusion of small bowel transplantation reflects rejection. Transplantation 55: 477–479
8. Kalland T, Alm G, Stålhandske T (1985) Augmentation of mouse natural killer cell activity by LS 2616, a new immunomodulator. J Immunol 134: 3956–3961
9. Koren R, Liberman UA, Maron L, Novogrodsky A, Ravid A (1989) 1,25-Dihydroxyvitamin D_3 acts directly on human lymphocytes and interferes with the cellular response to interleukin-2. Immunopharmacology 18: 187–194
10. Larsson E-L, Joki A, Stålhandske T (1987) Mechanism of action of the new immunomodulator LS 2616 on T cell responses. Int J Immunopharmacol 9: 425–431
11. Lemire JM, Archer DC (1991) 1,25-Dihydroxyvitamin D_3 prevents in vivo induction of murine experimental autoimmune encephalomyelitis. J Clin Invest 87: 1103–1107
12. Lemire JM, Adams JS, Kermani-Arab V, Bakke AC, Sakai R, Jordan SC (1985) 1,25-Dihydroxyvitamin D_3 suppresses human T helper/inducer lymphocyte activity in vitro. J Immunol 134: 3032–3035.
13. Lemire JM, Archer DC, Khulkarni A, Ince A, Uskokovic MR, Stepkowski S (1992) Prolongation of the survival of murine cardiac allografts by the vitamin D_3 analogue 1,25-dihydroxy-Δ^{16}-cholecalciferol. Transplantation 54: 762–763
14. Olausson M, Mjörnstedt L, Lindholm L, Brynger H (1984) Non-suture organ grafting to the neck vessels in rats. Acta Chir Scand 150: 463–467
15. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC (1983) 1,25-Dihydroxyvitamin D_3 receptors in human leukocytes. Science 221: 1181–1183
16. Reichel H, Koeffler HP, Tobler A, Norman AW (1987) 1α,25-Dihydroxyvitamin D_3 inhibits γ-interferon synthesis by normal human peripheral blood lymphocytes. Proc Natl Acad Sci USA 84: 3385–3389
17. Rigby WFC, Stacy T, Fanger MW (1984) Inhibition of T lymphocyte mitogenesis by 1,25-dihydroxyvitamin D_3 (calcitriol). J Clin Invest 74: 1451–1455
18. Rigby WFC, Noelle RJ, Krause K, Fanger MW (1985) The effects of 1,25-dihydroxyvitamin D_3 on human T lymphocyte activation and proliferation: a cell cycle analysis. J Immunol 135: 2279–2286
19. Rigby WFC, Denome S, Fanger MW (1987) Regulation of lymphokine production and human T lymphocyte activation by 1,25-dihydroxyvitamin D_3. Specific inhibition at the level of messenger RNA. J Clin Invest 79: 1659–1664
20. Tabata T, Suzuki R, Kikunami K, Matsushita Y, Inoue T, Inoue T, Okamoto T, Miki T, Nishizawa Y, Morii H (1986) The effect of 1α-hydroxyvitamin D_3 on cell-mediated immunity in hemodialyzed patients. J Clin Endocrinol Metab 63: 1218–1221
21. Tabata T, Shoji T, Kikunami K, Matsushita Y, Inoue T, Tanaka S, Hino M, Miki T, Nishizawa Y, Morii H (1988) In vivo effect of 1α-hydroxyvitamin D_3 on interleukin-2 production in hemodialysis patients. Nephron 50: 295–298
22. Tengblad A (1980) Quantitative analysis of hyaluronate in nanogram amounts. Biochem J 185: 101–105
23. Tobler A, Gasson J, Reichel H, Norman AW, Koeffler HP (1987) Granulocyte-macrophage colony-stimulating factor. Sensitive and receptor-mediated regulation by 1,25-dihydroxyvitamin D_3 in normal human peripheral blood lymphocytes. J Clin Invest 79: 1700–1705
24. Tsoukas CD, Watry D, Escobar SS, Provvedini DM, Dinarello CA, Hustmyer FG, Manolagas SC (1989) Inhibition of interleukin-1 production by 1,25-dihydroxyvitamin D_3. J Clin Endocrinol Metab 69: 127–133
25. Vieth R (1990) The mechanisms of vitamin D toxicity. Bone Miner 11: 267–272
26. Wallander J, Holtz A, Larsson E, Gerdin B, Läckgren G, Tufveson G (1988) Small-bowel transplantation in the rat with a nonsuture cuff technique: technical and immunological considerations. Transpl Int 1: 135–139
27. Wanders A, Larsson E, Gerdin B, Tufveson G (1989) Abolition of the effect of cyclosporine on rat cardiac allograft rejection by the new immunomodulator LS-2616 (Linomide). Transplantation 47: 216–217
28. Wientroub S, Winter CC, Wahl SM, Wahl LM (1989) Effect of vitamin D deficiency on macrophage and lymphocyte function in the rat. Calcif Tissue Int 44: 125–130