Volume 333, number 1,2, 83–88 FEBS 13142 October 1993

Uterine cells other than stromal decidual cells are required for 1,25-dihydroxyvitamin D_3 production during early human pregnancy

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Received 23 August 1993

Human decidual cells are known to produce 1,25-$(OH)_2D_3$ at the end of pregnancy, the present study evaluates this capacity, and the part played by stromal decidual cells, in early pregnancy. Cells were obtained from nine human decidua by aspiration or curettage during early pregnancy (7–10 weeks), separated on Ficoll-Paque and plastic adherence, and incubated for 1 h with 25-$(OH)D_3$. Incubation medium and cells were extracted and chromatographed on two successive HPLC systems. The cells examined were of both physiological and pathological (ectopic pregnancy) origin. Endometrial cells obtained in four non-pregnant situations (myomas) were also studied to determine whether the 1,25-$(OH)_2D_3$ synthesis by the uterus is associated with the appearance of decidual cells. Results show that human decidual cells from early pregnancy convert 25$(OH)D_3$ (2.5 nM or 2.5 μM) into a metabolite with the physicochemical characteristics of synthetic 1,25-$(OH)_2D_3$. This ability is shared by cells isolated during early pregnancy, whether physiological or ectopic (tubal pregnancy). Non-adherent cells, which include mainly stromal decidual cells, are less able to produce 1,25-$(OH)_2D_3$ than are the adherent cells, suggesting that macrophages, granulocytes or as yet unidentified cell types are required for the 1,25-$(OH)_2D_3$ production by decidual tissue during early human pregnancy. In addition, one out of four experiments with non-pregnant endometrial cells could produce 1,25-$(OH)_2D_3$ suggesting that, although not the rule in the non-pregnant state, in vitro production of 1,25-$(OH)_2D_3$ by uterine cells can be found in the absence of decidual cells.

Human early pregnancy; Myoma; Decidual cell; Uterine cell; In vitro 1,25-$(OH)_2D_3$ production

1. INTRODUCTION

The kidney has long been considered to be the exclusive site at which 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), the hormonal active form of vitamin D_3, is synthesized [1–4]. This concept is based on experimental results showing that 1,25-$(OH)_2D_3$ is no longer formed after bilateral nephrectomy [1]. However, this is clearly not the case during pregnancy, as rat maternal blood contains 1,25-$(OH)_2D_3$ even after experimental bilateral nephrectomy [5,6], and as foeto-placental tissues from both animals and humans synthesize 1,25-$(OH)_2D_3$ in vitro [7–13].

Studies on the ability of the human uterine cells to synthesize 1,25-$(OH)_2D_3$ have so far been mostly limited to the late pregnancy period [9–13], and/or have involved incubations with whole tissue homogenates [8,9,12,13]. The location and characterization of the cells responsible for the 1,25-$(OH)_2D_3$ synthesis in the feto-placental unit at this time of pregnancy remains a fertile field for debate [8–13]. To date, an in vitro 1,25-$(OH)_2D_3$ production has been observed during incubations of placenta homogenate or mitochondria [9,12,13]. But this 1,25-$(OH)_2D_3$ production appears to require high substrate concentrations (0.1–100 μM of 25-$(OH)D_3$), and may be dependent upon an enzyme system different from that described in kidney mitochondria [13]. In contrast, cultured decidual tissue or cell produce 1,25-$(OH)_2D_3$ when incubated with 25-$(OH)D_3$ concentrations as low as 0.6–6 nM [8,10,11], with a K_m of 88 nM [14], similar to that reported for cultured kidney cells [14], and for human pulmonary macrophages from patients with sarcoidosis, a classical extrarenal site of 1,25-$(OH)_2D_3$ production [15]. Thus, cells of decidual origin may be a source, even if not a major one [16], of maternal circulating 1,25-$(OH)_2D_3$, the concentration of which is elevated during late pregnancy [17–19].

This study evaluates the ability of decidual cells obtained during early pregnancy to synthesize 1,25-$(OH)_2D_3$ in vitro. The cells examined were of both physiological and pathological (ectopic pregnancy) origin, since decidualization of the endometrium occurs as well in case of uterine or ectopic egg implantation. Several studies clearly indicate the heterogeneity of the cell populations present in early gestational human decidua [20–22]. Two cell populations were studied after isolation by Ficoll-Paque and a further fractionation by plastic adherence, a technique which has been shown to separate populations containing mainly stromal decidual cells

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from populations containing a number of other cell types including macrophages [20]. Endometrial cells obtained in non-pregnant pathological situations were also studied to determine whether the 1,25-$(OH)_2D_3$ synthesis by the uterus is associated with the appearance of decidual cells.

2. MATERIALS AND METHODS

2.1. *Tissues*

Decidual tissue was collected from nine pregnant uteri at presumably 7 to 10 weeks of gestational age after mild aspiration and curettage for elective termination of physiological pregnancy (samples 1–8), and by curettage during surgical treatment of ectopic tubal pregnancy (sample 9). Special care was taken to avoid contamination by trophoblastic cells.

Four samples of non-pregnant endometrial tissues were obtained from uteri after surgical hysterectomy for single (sample 10) or multiple myomas (samples 11–13) and with (samples 12,13) or without (samples 10,11) moderate inflammatory infiltrates. The endometrial samples were taken from apparently unaffected parts of the uterus from non-cyclic (sample 10), or cyclic (first part: sample 11; second part, samples 12 and 13) women aged 36–49 years.

Endometrial and decidual tissues were immediately transferred into sterile ice-cold minimal essential medium (MEM, Gibco, France).

2.2. *Disaggregation of cells*

The method used was that described by Parhar et al. [20]. Briefly, tissues were washed in ice cold MEM, freed from blood clots, mucus, and any chorionic villi, washed again to minimize contamination by trophoblastic cells, and minced finely with a scalpel. Minced tissue (1 g) was digested in 10 ml calcium and magnesium-free phosphate-buffered saline containing 0.1 mg/ml collagenase type IA (Sigma, France) for 60 min at 37°C. Cells were collected by centrifugation (5 min at 900 × *g*) and the undigested tissues were incubated with collagenase for two further 30 min periods. The cells were washed with Dulbecco's modified Eagle's medium (DMEM) (Gibco, France) containing 10% fetal calf serum (FCS, Seromed, France). In some experiments, RPMI1640 culture medium (Gibco, France), was used instead of DMEM. The suspension was then filtered through sterile cheesecloth (60 μm nylon mesh NY_{60}) and the cells separated by centrifugation for 20 min on a Ficoll-Paque density gradient at 3500 rpm (density 1.078, Pharmacia). The cells at the interface (Ficoll-medium) were removed, washed, counted, and resuspended in culture medium RPMI1640 or DMEM + 10% FCS at 10^6 cells/ml. The viability of the recovered fresh cells, as assessed by Trypan blue exclusion, was 94%. Cells (10^6/ml) were then incubated overnight in glass Lab-Tek chamber slides (Miles laboratory, Naperville, IL, USA) at 37°C, under 95% air, 5% CO_2. Adherent and, in some experiments, non-adherent cells isolated from the decidual or endometrial tissues were kept after the overnight culture for further analysis.

2.3. *In vitro metabolism of 25(OH)D_3*

The capacity of adherent and non-adherent cells to metabolize 25(OH)D_3 in vitro was assessed by incubating 10^6 viable cells for 60 min (at 37°C, 95% air, 5% CO_2) in glass Lab-Tek chamber slides containing 2 ml serum-free DMEM or RPMI1640.

10 μl of ethanol were then added which contained 50 nCi [26,27-^{3}H]-25(OH)D_3 (20 Ci/mmol, Radiochemical Centre, Amersham, UK; final concentration 2.5×10^{-9} M). After 60 min, cells were scraped free and the medium and cells were transferred to glass tubes. Two ml methanol plus 2 ml chloroform were added to each sample. After 24 h at 4°C the chloroform phase was removed, dried under N2, and the residue redissolved in chromatographic solvent. Before chromatography, 80 ng unlabeled synthetic 1,25-$(OH)_2D_3$ (Hoffman-La Roche, Inc., Nutley, NJ) were added to extracts from cells as a marker of the 1,25-$(OH)_2D_3$ elution position detectable at 254 nm. To quantify 1,25-$(OH)_2D_3$ production, samples were chromatographed on a direct phase HPLC system (Beckman, Berkeley, CA, USA) using an Ultrasphere Si column (5 μm, 4.6 × 250mm, Altex, Berkeley, CA, USA) and a 92:8 *n*-hexane/isopropanol solvent (flow rate 1.6 ml/min). The metabolites comigrating with the simultaneously injected synthetic 1,25-$(OH)_2D_3$ were rechromatographed on the same HPLC system using a 95:5 methylene-chloride/isopropanol solvent (flow rate 1 ml/min) as previously described [23]. The rate of conversion of [^{3}H]25(OH)D_3 into [^{3}H]1,25-$(OH)_2D_3$ was determined by calculating the percentage of total radioactivity with an appropriate elution profile after the two successive separations. Results are expressed as fmol/10^6 cells per 1 h, assuming that the specific activity of the product was the same as that of the substrate. Thus, conversion of 1% substrate corresponds to the production of 25 fmol [^{3}H]1,25-$(OH)_2D_3$.

2.4. *Binding of putative 1,25-$(OH)_2D_3$ to 1,25-$(OH)_2D_3$ receptors*

Other decidual cell incubations were made in the presence of 1 μg unlabeled 25(OH)D_3 (Roussel-Uclaf Laboratories, Paris, France; final concentration 1×10^{-6} M) instead of 2.5×10^{-9} M [^{3}H]25(OH)D_3. The putative 1,25-$(OH)_2D_3$ produced during these incubations was purified as above with one exception, cell and medium extracts were cochromatographed on HPLC with 2 nCi synthetic [^{3}H]1,25-$(OH)_2D_3$ as a radioactive marker of the 1,25-$(OH)_2D_3$ elution position. The putative purified 1,25-$(OH)_2D_3$ was tested for its ability to compete with synthetic [^{3}H]1,25-$(OH)_2D_3$ for binding to the 1,25-$(OH)_2D_3$ receptors in chick intestinal cytosol [24]. The metabolite produced was quantified from its absorbance at 254 nm.

3. RESULTS

Human decidual and endometrial cells converted 2.5 nM [26,27] [^{3}H]25(OH)D_3 into polar radioactive derivatives when incubated for 1 h in serum-free RPMI1640 or DMEM (Figs. 1 and 2).

We have been interested in the metabolite that coeluted with synthetic 1,25-$(OH)_2D_3$ in the two sequential HPLC chromatographic systems. Further analysis of this putative 1,25-$(OH)_2D_3$ was made after incubation of the cells with larger amounts of 25-(OH)D_3 (1 μM). This confirmed the identification of the metabolite as 1,25-$(OH)_2D_3$. Decidual cells produced a metabolite detected by UV absorbance at 254 nm which coeluted with synthetic [^{3}H]1,25-$(OH)_2D_3$ in the two sequential HPLC chromatographic systems. This metabolite also had the same capacity as synthetic 1,25-$(OH)_2D_3$ to compete with [^{3}H]1,25-$(OH)_2D_3$ for binding to the chick intestinal cytosol receptor (Fig. 3, Table I). The only known vitamin D metabolite with these characteristics is 1,25-$(OH)_2D_3$ [25–27].

It was necessary to chromatograph samples on two successive systems in order to isolate 1,25-$(OH)_2D_3$ from another 25-(OH)D_3 derivative which was produced by all tested cell populations. This latter derivative could be (5*E*)-19-nor-10-oxo-25(OH)D_3 [26,27]. It was present in the 26–29 ml elution fractions containing the synthetic 1,25-$(OH)_2D_3$ after chromatography in the first HPLC system, but was detected in the 9–10 ml fraction, before 1,25-$(OH)_2D_3$, in the second HPLC system (Figs. 1 and 2). Moreover, incubations of decidual cells with 2.5 μM 25-(OH)D_3 showed that this derivative did not absorb UV light at 254 nm, and did not bind to

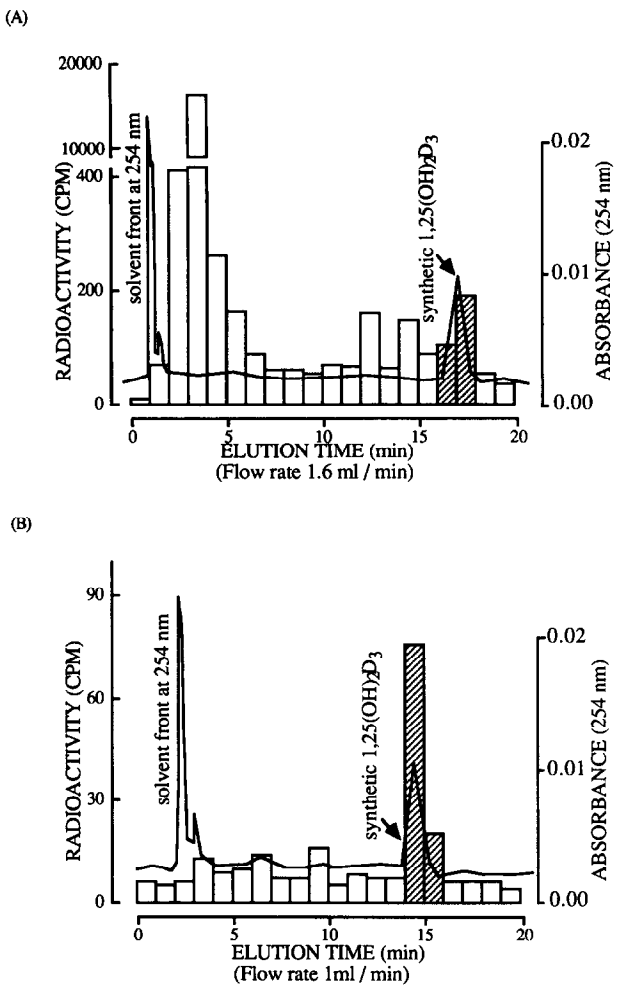

Fig. 1. Typical high-pressure liquid chromatography elution profiles of the radioactivity extracted from human decidual cells incubated with 2.5 nM [^{3}H]25(OH)D_3, and the absorbance at 254 nm of 80 ng unlabeled synthetic 1,25$(OH)_2D_3$ added to extracts before chromatography. (A) First chromatography using a 92:8 *n*-hexane/isopropanol solvent, the elution position of (5*Z*)-19-nor-10-oxo-25(OH)D_3 in this system is 22–24 ml, that of (5*E*)-19-nor-10-oxo-25(OH)D_3 is 26–29 ml; (B) chromatography using a 95:5 methylene-chloride/isopropanol solvent of the 1,25$(OH)_2D_3$ region isolated from (A), the elution position of (5*Z*)-19-nor-10-oxo-25(OH)D_3 in this second HPLC system is 6–7 ml, that of (5*E*)-19-nor-10-oxo-25(OH)D_3 is 9–10 ml. Hatched bars: radioactivity coeluted with synthetic 1,25$(OH)_2D_3$. Note that most of the radiocativity eluting in the 1,25$(OH)_2D_3$ region in the first HPLC system also coelutes with 1,25$(OH)_2D_3$ in the second HPLC system.

1,25-$(OH)_2D_3$ receptors in chick intestinal cytosol (samples 6 and 7, data not shown).

The incubation medium influenced the ability of the cells to convert [26,27-^{3}H]25OHD_3 into [^{3}H]1,25-$(OH)_2D_3$. This ability was not detectable in the 6 uterine cell populations tested in RPMI (below 1.5 fmol/10^6 cells/h). It was detectable in 10 of the 13 uterine cell populations tested in DMEM (Table I).

Adherent human decidual cells from all eight samples obtained during physiological early pregnancy converted [^{3}H]25(OH)D_3 into [^{3}H]1,25-$(OH)_2D_3$ (1.75–11 fmol/10^6 cells/h), as did the adherent decidual cells obtained from the patient with an ectopic pregnancy (Table I). In contrast, only one of the four samples of endometrial adherent cells from non-pregnant patients with myomas converted [^{3}H]25(OH)D_3 into [^{3}H]1,25-$(OH)_2D_3$ (Table I). The three other endometrial samples converted [^{3}H]25(OH)D_3 only into the (5*E*)-19-nor-10-oxo-25(OH)D_3-like derivative (Fig. 2).

Non-adherent decidual cells had a 3- to 11-fold lower capacity to convert [^{3}H]25-(OH)D_3 into [^{3}H]1,25-

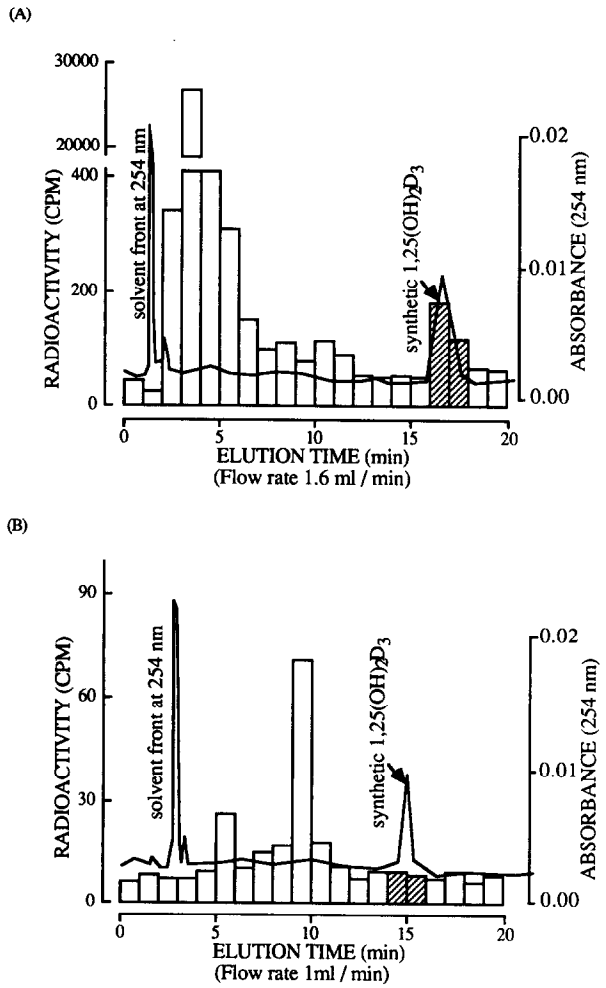

Fig. 2. Typical high-pressure liquid chromatography elution profiles of the radioactivity extracted from human non-pregnant endometrial cells incubated with 2.5 nM [^{3}H]25(OH)D_3, and the absorbance at 254 nm of 80 ng unlabeled synthetic 1,25$(OH)_2D_3$ added to extracts before chromatography. (A) First chromatography using a 92:8 *n*-hexane/isopropanol solvent, the elution position of (5*Z*)-19-nor-10-oxo-25(OH)D_3 in this system is 22–24 ml, that of (5*E*)-19-nor-10-oxo-25(OH)D_3 is 26–29 ml; (B) chromatography using a 95:5 methylene-chloride/isopropanol solvent of the 1,25$(OH)_2D_3$ region isolated from (A), the elution position of (5*Z*)-19-nor-10-oxo-25(OH)D_3 in this second HPLC system is 6–7 ml, that of (5*E*)-19-nor-10-oxo-25(OH)D_3 is 9–10 ml. Hatched bars: radioactivity coeluted with synthetic 1,25$(OH)_2D_3$. Note that most of the radiocativity eluting in the 1,25$(OH)_2D_3$ region in the first HPLC system does not coelute with 1,25$(OH)_2D_3$ but migrates to the (5*E*)-19-nor-10-oxo-25(OH)D_3 region in the second HPLC system.

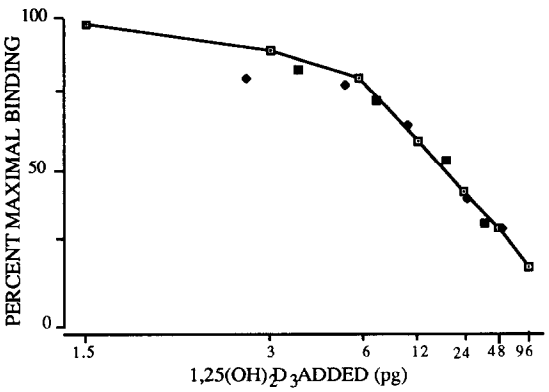

Fig. 3. Characterization of $1,25(OH)_2D_3$ produced by human decidual cells incubated with 2.5 μM unlabeled $25(OH)D_3$. The metabolite coeluting with synthetic [^{3}H]$1,25\text{-}(OH)_2D_3$ on the two successive HPLC systems (see Fig. 1) was isolated. Its ability to compete with the binding of [^{3}H]$1,25(OH)_2D_3$ to its chick intestinal receptor (closed symbols) was compared with that of known amounts of synthetic $1,25(OH)_2D_3$ (open symbols).

$(OH)_2D_3$ than the adherent cells from the same uterine samples (Fig. 4).

4. DISCUSSION

Cells obtained from human decidua by aspiration or curettage during early pregnancy (7–10 weeks) and separated on Ficoll-Paque convert [^{3}H]$25(OH)D_3$ into a metabolite with the physicochemical characteristics of synthetic $1,25\text{-}(OH)_2D_3$: the synthetic and biosynthetic $1,25\text{-}(OH)_2D_3$ coeluted in the two HPLC chromatographic systems used, had similar UV absorbance at 254 nm, and competed identically with [^{3}H]$1,25\text{-}(OH)_2D_3$ for binding to chick intestinal cytosol receptor.

Following the reports of Weisman et al. [5], Gray et al. [6], and Tanaka et al. [7], kidney is no longer considered to be the sole site of $25\text{-}(OH)D_3$-1 α-hydroxylation during pregnancy. Several studies have reported that the term human feto-placental unit can produce $1,25\text{-}(OH)_2D_3$ [8–13]. But the location and characterization of the cells responsible for this synthesis in the feto-placental unit is not clear. Human trophoblastic tissue homogenates produce $1,25\text{-}(OH)_2D_3$ in vitro [9,12,13], but not under all experimental conditions [8], and only when high substrate concentrations are used, 0.1–100 μM. Moreover, the K_m of this production is higher, 570 nM [12], than that reported for $1,25\text{-}(OH)_2D_3$ production by kidney cells, 125 nM [15], and trophoblastic tissue may produce $1,25\text{-}(OH)_2D_3$ in vitro by a mechanism different from the classical one involving the cytochrome P450 mixed function oxidase [13].

As for human decidual tissue, term decidua basalis incubated in vitro with high substrate (100 μM) concentrations cannot produce $1,25\text{-}(OH)_2D_3$ in vitro [9]. But term decidua parietalis express this capacity when incubated with nM $25\text{-}(OH)D_3$ concentrations [8,10,11] with a K_m, 88 nM [11], similar to that reported for kidney cells [15]. In the present study, decidual cells expressed the capacity to form $1,25\text{-}(OH)_2D_3$ as early as the 7–10th week of pregnancy and this ability was similar to that reported for human decidual cells at the end of pregnancy. Indeed, 2–11 fmoles / 10^6 cells / h [^{3}H]$1,25\text{-}(OH)_2D_3$ was produced by incubation of early pregnancy decidual cells with 2.5 nM [^{3}H]$25\text{-}(OH)D_3$, while full-term decidual cells produce 33–168 fmol/10^6 cells/16 h with similar concentrations of [^{3}H]$25\text{-}(OH)D_3$, 6 nM [10]. Moreover, incubations of early pregnancy decidual cells with 2.5 μM $25\text{-}(OH)D_3$ produced 4.5–5 pmol/10^6 cells/h $1,25\text{-}(OH)_2D_3$, and the V_{max} for such production by late pregnancy decidual cells is 3 ± 0.4 pmol/10^6 cells/h [11]. The $1,25\text{-}(OH)_2D_3$ production by both early and late pregnancy decidual cells appears to be lower than that obtained with cultured chick kidney cells, about 50 pmol/10^6cells/h [15], but it is similar to that reported for cultured human pulmonary macrophages from patients with sarcoidosis, 0.4–2.5 pmol/10^6 cells/h [16]. The results of the present study confirm the ability of cells from human decidual tissue to produce $1,25\text{-}(OH)_2D_3$ in vitro, when incubated with 2.5 or 2500 nM $25\text{-}(OH)D_3$ concentrations, and show that this ability is shared by cells isolated during early pregnancy, whether physiological or even ectopic (tubal pregnancy).

The control of extrarenal $1,25\text{-}(OH)_2D_3$ synthesis appear to be different from that of the renal synthesis of

Table I

$1,25\text{-}(OH)_2D_3$ production by adherent human decidual cells and non-pregnant endometrial cells (fmol/10^6 cells/h)

Experiments	$1,25\text{-}(OH)_2D_3$ produced during incubation with	
	2.5 nM [^{3}H]$25\text{-}(OH)D_3$[a]	2.5 μM $25\text{-}(OH)D_3$[b]
Human decidua		
1*	1.75 (1.5– 2.0)	
2*	11.00 (7.0–15.0)	
3	2.00	
4	2.02	
5	5.31	
6	2.62	
7	–	4500
8	–	5000
9[c]	5.50	
Non-pregnant uturus		
10	< 1.50	
11	< 1.50	
12	< 1.50	
13*	7.00 (6.4– 7.7)	

[a] $1,25\text{-}(OH)_2D_3$ production was estimated from the amount of radioactivity coeluting with synthetic $1,25\text{-}(OH)_2D_3$ on the 2 HPLC systems. It was assumed that [^{3}H]$1,25\text{-}(OH)_2D_3$ produced and the [^{3}H]$25\text{-}(OH)D_3$ substrate had identical specific activities.

[b] $1,25\text{-}(OH)_2D_3$ production was quantified by direct UV absorbance at 254 nm, and, measured more precisely by the radioreceptor assay shown in Fig. 3.

[c] Ectopic tubal pregnancy.

*Mean value, and range of duplicate incubations.

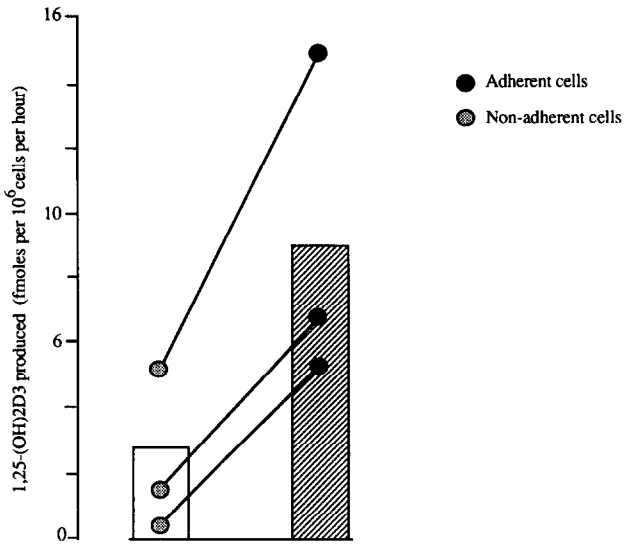

Fig. 4. Comparison of the capacity of adherent and non-adherent decidual cells to convert [^{3}H]25(OH)D_3 into [^{3}H]1,25(OH)$_2D_3$. Cells were obtained from three samples from decidual tissues and separated by Ficoll-Paque and plastic adherence as described in section 2. 1×10^6 cells were incubated with 2.5 nM [^{3}H]25(OH)D_3 in serum-free DMEM medium, and the amount of [^{3}H]1,25(OH)$_2D_3$ produced was determined as described in section 2. Bars represent the mean of individual values for each cell population.

this metabolite [30–33]. Delvin et al. showed that neither inorganic phosphate salts (0–4 mM), nor parathyroid hormone had any acute effect on 1,25-(OH)$_2D_3$ production by human term decidual cells [11]. Hollis et al. suggested that the enzyme system responsible for this production in decidual cells may be, like for the trophoblastic portion of the human placenta, different from that present in the kidney mitochondria [13]. Such a hypothesis has not been tested yet, but above mentioned data of kinetic studies suggest similarities between 1,25-(OH)$_2D_3$ productions by human decidual cells and kidney mitochondria [8,10,11], which are not found with term human placenta [12,13]. In our experimental conditions, 1,25-(OH)$_2D_3$ production by human adherent decidual cells depends on the incubation medium used. It was detectable in DMEM but not in RPMI 1640 medium. As these media differ mainly in their inorganic phosphate salt concentrations, respectively 0.9 mM for DMEM and 5 mM for RPMI1640, phosphorus may regulate 1,25-(OH)$_2D_3$ production by human decidual cells, at least during early pregnancy.

Parallel studies of adherent and non-adherent cells isolated from the same early gestational decidual tissues provide further indications of the characteristics of the cells responsible for 1,25-(OH)$_2D_3$, production. It is known that the cell populations isolated from the decidua depend on the isolation method used, bone-marrow derived cells are preferentially selected by enzyme digestion whereas mechanical disaggregation isolates cell populations in proportions which are more representative of those found in the intact tissue [22]. Cell populations from early gestational human decidua can be fractionated by Ficoll-Paque and further fractionated by plastic adherence into adherent and non-adherent cells. Non-adherent cells contain mainly stromal decidual cells (96%), almost no macrophages and a small number of other cell types, including 1% trophoblastic cells. Adherent cells include, besides a similarly small amount of trophoblastic cells (1%), more macrophages (25%) and granulocytes (9%) and less stromal type decidual cells (about 60%) [20]. Using this cell fractionation technique, we found that non-adherent cells were less able to produce 1,25-(OH)$_2D_3$ than were the adherent cells. This suggests that macrophages, granulocytes or as yet unidentified cell types are required for the 1,25-(OH)$_2D_3$ production by decidual tissue during early human pregnancy. These cell types may be the source of 1,25-(OH)$_2D_3$ or they may secrete local factors which stimulate the 1,25-(OH)$_2D_3$ production by stromal decidual cells.

Finally, the studies on human endometrial cells from non-pregnant pathological uteri suggest that, unlike during pregnancy, 1,25-(OH)$_2D_3$ production by human uterine endometrial cells is not the rule in the non-pregnant state. But, as one of the four samples tested did produce 1,25-(OH)$_2D_3$, perhaps some uterine pathologies can lead to the production of 1,25-(OH)$_2D_3$ by the uterus. Hartwell et al. [34] recently reported that women with endometriosis have significantly higher serum 1,25-(OH)$_2D_3$ concentrations than healthy women. Our in vitro findings suggest that such elevated 1,25-(OH)$_2D_3$ concentrations may be due to the local uterine synthesis of this metabolite and that uterine cells other than stromal decidual cell can express a 25-(OH)D_3-1 α-hydroxylase activity.

In conclusion, human decidual cell populations isolated by Ficoll-Paque and plastic adherence from early physiological and pathological (ectopic) pregnancy uteri can synthesize 1,25-(OH)$_2D_3$. Although not the rule, endometrial cells isolated from cases of non-pregnant uterine pathologies are also able to produce 1,25-(OH)$_2D_3$. Cell populations other than stromal decidual cells are required for this synthesis.

Acknowledgements: We thank the members of the Obstetrical department, Clinique Universitaire Baudelocque, Paris, and of the Pathological Anatomy department, Hôpital Saint Vincent de Paul, Paris, for providing decidual and endometrial tissues.

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