Alternatively Activated Macrophages are the Primary Retinoic Acid Producing Cells in Human Decidua

Augustine Rajakumar
Emory University School of Medicine
Maureen A. Kane
University of Maryland
Jianshi Yu
Univeristy of Maryland
Jace W. Jones
University of Maryland
Hongyan Qu
Emory University School of Medicine
Martina Badell
Emory University School of Medicine
Robert N. Taylor
University of Utah School of Medicine
Neil Sidell (nsidell@emory.edu)
Emory University School of Medicine

Research Article

Keywords: Retinoic acid, decidua, M2 macrophages, pregnancy

Posted Date: October 30th, 2019

DOI: https://doi.org/10.21203/rs.2.16738/v1

Abstract

In situ production and metabolism of all-trans retinoic acid (RA) in decidual tissue are critically important for endometrial stromal differentiation, embryo implantation, and healthy placentation. However, the cellular source(s) of RA in this tissue has yet to be determined. To identify the primary RA-producing cells in human term decidua, we isolated cells from decidua basalis of delivered placenta and quantified cellular retinal dehydrogenase (RALDH) activity, a major biosynthetic enzyme whose activity determines the synthesis of RA from retinol, using an Aldefluor assay and flow cytometry. RA production in decidual tissue and sorted cell subpopulations was evaluated by liquid chromatography-tandem mass spectrometry. $CD14^+$ cells (macrophages/monocytes) showed >4-fold higher RALDH activity than stromal cells ($CD10^+$), T-cells ($CD3^+$), or non-T lymphocytes (CD3-negative). $CD11c^+$ cells that did not co-express CD14 showed about one-third the RALDH activity of their CD14 co-expressing counterparts. The highest RALDH activity was found in “alternatively activated” M2 macrophages delineated by the simultaneous expression of CD14 and CD163. The greater RA synthesizing capacity of M2 versus $CD14^+$CD163-ve (M1) cells was confirmed by direct quantitation of RA biosynthesis from retinol. RA levels in whole decidua correlated with M2 cell density but not with stromal cell ($CD10^+$) number, the major cell type comprising the decidua. These results identified M2 monocyte/macrophages as the primary source of RA in human term decidua. This finding may have implications for certain pregnancy complications that are known to be associated with reduced numbers of decidual M2 cells.

Author note: This manuscript has been accepted for publication in the journal Reproductive Sciences

Introduction

Vitamin A, through its active metabolite all-trans retinoic acid (RA), is essential to the homeostasis of mucosal tissue and regulation of innate adaptive immunity [1–4]. In this regard, the most extensively studied compartment is the gastro-intestinal tract (GIT) where tolerance to a plethora of foreign antigens (e.g. commensal microorganisms) is mediated by regulatory T cells (Tregs) that are in part regulated by RA produced and secreted by specialized dendritic cells [5,6]. In several inflammatory bowel diseases (e.g. Crohn’s disease, ulcerative colitis), both intestinal Treg density and RA production are suppressed compared to controls [7,8].

Like the GIT, hemochorial placentation in the uterus requires immune tolerance to the semi-allogeneic antigens of the fetus during pregnancy; this topic has been a subject of intense interest [9]. Over the years, studies aimed at understanding this phenomenon have focused on leukocyte subpopulations in the maternal decidua that may account for suppressing anti-allogeneic rejection [9–11]. In analogy to GIT tolerance, enrichment of decidual Tregs has been implicated as playing an indispensable role in sustaining maternal tolerance to the fetus [12]. Interestingly, the density of this RA-regulated cell population

is reduced in various pregnancy-related syndromes associated with a proinflammatory phenotype, including fetal growth restriction, pre-term labor, and preeclampsia [12–14].

In addition to its immune regulatory function, recent reports have shown that *in situ* RA production plays an important role in optimal endometrial stromal cell decidualization via regulation of gap junctions to form competent decidua for trophoblast attachment and implantation, and placental vascularization [15–17]. Numerous studies have demonstrated that the expression of RA nuclear receptors, cellular RA-binding proteins, and RA-catabolizing enzymes are tightly regulated in the differentiating endometrium during the ovarian cycle and during the phase of blastocyst nidation [18–20]. Despite its overall importance, the main source(s) of RA production in the decidua has not been identified. In this study, we have determined that the main cellular source of RA synthesis in term decidua are alternatively activated (M2) macrophages [21–23]. This finding may have implications with respect to reproductive disorders associated with pregnancy-related proinflammatory conditions.

Materials And Methods

Source of tissue. Decidua basalis tissue was collected from 20 delivered placentas according to a protocol approved by the Emory Institutional Review Board (IRB00078902) as part of another study [24]. Of these, 8 placentas were from spontaneous vaginal deliveries (S/D) while 12 were from planned cesarean section deliveries (C/S). Signed informed consent was obtained from all subjects. We included non-smoking, English-speaking women above 18 years old and excluded women with a preexisting chronic condition that could complicate pregnancy (diabetes, pregestational or chronic hypertension, congenital heart disease, autoimmune disorder).

Decidual cell isolation and flow cytometry. The decidual tissue adherent to the maternal surface of the placenta was dissected and processed for cell isolation within 30 minutes of delivery. Per published protocols, tissue was finely minced and enzymatically digested using 0.1% collagenase and filtered through 100 micron sieves [25]. The collected cells were resuspended and layered on Ficoll-Paque (GE Healthcare, Uppsala Sweden). Following centrifugation (2500 RPM/20 minutes), the buffy coat was collected to obtain mononuclear cells for this study. Flow cytometry of the mononuclear cell populations was performed on a FACScalibur (BD Biosciences) using specific fluorochrome-conjugated antibodies (BD Biosciences) directed against CD45 (leukocyte common antigen), CD14 (monocytes, macrophages), CD11c (dendritic cells, some monocytes), CD3 (T-lymphocytes), and CD10 (decidual stromal cells) [26,27]. Isotype control antibodies were used at the same dilution as their counterparts. For each experiment, 100,000 events were collected and analyzed using the Flowjo 10 program. The data were expressed as mean percent positive cells of the appropriately gated population.

RALDH levels and retinoid quantitation. RALDH activity was determined using the Aldefluor® assay (StemCell technology, Vancouver, Canada), as described [28]. Briefly, cell suspensions ($1x10^6$ cells/ml) were incubated for 45 minutes at 37°C in Aldefluor assay buffer containing activated Aldefluor substrate in the

presence or absence of the RALDH inhibitor diethylaminobenzaldehyde (DEAB). The cells were subsequently stained with specific antibodies, washed, resuspended in Aldefluor assay buffer and analyzed by flow cytometry in a FACScalibur (BD Biosciences). RA concentrations were assessed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described previously [29–31]. Briefly, cells or tissue samples were homogenized in ground glass homogenizers in 1.0 mL saline (0.9% NaCl). Extraction of retinoids was performed under yellow lights using a two-step liquid-liquid extraction that has been described in detail previously using 4,4-dimethyl-RA as an internal standard [29,30]. Levels of RA were determined by liquid chromatography-multistage tandem mass spectrometry (LC-MRM^3) which is an LC-MS/MS method utilizing two distinct fragmentation events for enhanced selectivity [30]. RA was measured using a Shimadzu Prominence UFLC XR liquid chromatography system (Shimadzu, Columbia, MD) coupled to an AB Sciex 5500 QTRAP hybrid triple quadrupole mass spectrometer (AB Sciex, Framingham, MA) using atmospheric pressure chemical ionization (APCI) operated in positive ion mode as previously described [30]. RA in tissue and cells are expressed as mol/g protein and pmol/10^6 cells, respectively.

For determining RA biosynthesis from selected cell populations, the purified populations were obtained by fluorescence activated cytometry (FACSAria II), washed with PBS, and then cultured in serum-free medium in the presence of 2 μM retinol (ROL, Sigma Chemical Co.) or solvent control (dimethyl sulfoxide) for 6 h. The final concentration of dimethyl sulfoxide as solvent was always less than 0.1%. RA synthesized from ROL by the cells was quantified as above and cellular RA was expressed as pmol/10^6 cells.

Statistical Analysis. Statistical analysis was performed using GraphPad Software (San Diego, CA). Data are presented in scatter dot plots as median and interquartile range. Differences between groups were analyzed by Kruskal-Wallis test where $p < 0.05$ was considered statistically significant.

Results

Decidual cells from term placenta. Flow analysis of mononuclear (buffy coat) cells obtained from harvested decidua determined that (mean ± s.d.) 33 ± 9% were leukocytes (n = 10) and 44 ± 7% were stromal cells (n = 10) by positive expression of CD45 or CD10, respectively. By staining with mAb to CD14 (monocytes, macrophages), CD11c (dendritic cells, some monocytes) and CD3 (T-lymphocytes), we determined that the majority of CD14$^+$ and CD11c$^+$ cells fall into the “large cell” scatter gate (R2), while the lymphocytes fall into the “small cell” scatter gate (R1, Fig. 1). These scatter characteristics are consistent with corresponding cell populations found in peripheral blood [32] and were used to calibrate analysis of the CD14, CD11c, and CD3$^+$ subpopulations of dispersed decidual cells. Fig. 1 also shows the scatter characteristics of the CD10$^+$ stromal cell population. By subsequent gating on CD45 expression, we found that CD14 and CD11c were detected on 75 ± 13% (n = 10) and 39 ± 19% (n = 9) of the CD45$^+$ leukocyte population in R2, respectively, while CD3 was detected on 40 ± 7% of the CD45$^+$ cells in R1 (n = 4). The subpopulations of CD14$^+$ and CD11c$^+$ cells were not distinct as 64 ± 12% of CD11c$^+$ cells co-

expressed CD14 (n = 8). Thus, cells expressing either CD14 or CD11c (or both) comprise ~90% of the $CD45^+$ population in the large cell R2 scatter gate. The polarization state of $CD14^+$ cells was evaluated as to whether they were either classically (M1) or alternatively (M2) activated based upon expression of CD163 [22,33,34]. To this end, $CD14^+CD163^+$ M2 cells were found to be the dominant polarization state of CD14+ monocytes (75 ± 12%; n = 19). The mode of birth (N/P or C/S) did not significantly affect the distribution or phenotype characteristics of the decidual cells in our analyses. Overall, these percentages of leukocyte and stromal cell subpopulations from human decidual tissue are consistent with published reports [10,35,36].

Cellular RALDH activity. RA concentrations are regulated *in vivo* through the activity of the retinal dehydrogenase (RALDH) enzymes, which catalyze the oxidation of retinal to RA [1,4,37]. RALDH expression is limited to select cell types and, in some cases, inducible by various stimuli including RA itself [1,3,37]. To determine the major cellular sources of RA in term decidua, we utilized the Aldefluor® assay as previously reported to identify cells expressing high levels of RALDH [38]. As seen in Fig. 2, the cell populations showing the highest RALDH activity were $CD14^+$ and $CD11c^+$ cells. The similar concentration of RALDH activity in these two cell populations was not surprising since the majority of $CD11c^+$ cells co-express CD14. In contrast, levels of RALDH activity in stromal cells ($CD10^+$) or T lymphocytes ($CD3^+$), as well as CD3-negative non-T lymphocytes (CD3-ve), were <25% of that seen in $CD14^+$ and $CD11c^+$ cells. When $CD11c^+$ cells were analyzed according to their co-expression of CD14, we found that the subpopulation showing the highest RALDH activity segregated with positive co-expression of CD14 (Fig. 3). $CD14^+$ cells were then delineated by the simultaneous expression of CD14 and CD163. Results indicated that the M2 ($CD163^+$) subpopulation showed the highest level of RALDH activity, greater than 3-fold that of M1 (CD163-ve) macrophages (Fig. 4). The ability of the cell populations to synthesize RA was assessed by LC-MS/MS analysis following addition of 2 μM retinol for 6 hr under serum-free conditions [29,39,40]. Results validated the correlation between RALDH activity and the retinoid synthesizing capacity of the cell populations and confirmed the greater production of RA by M2 versus M1 cells. (Fig. 4 upper panel). To assess whether M2 cells, as major RA producers, are the primary source of RA production in decidual tissue, we compared the concentration of endogenous RA in whole decidual tissue with the percentage of M2 cells (M2/total cells x 100) obtained from the corresponding tissue. Fig. 5 shows a statistically significant correlation between these variables ($r = 0.89$, $P = 0.02$) supporting the contention that M2 cells are the primary RA source in the decidua. In contrast, there was no correlation between $CD10^+$ stromal cell number, the major cell type comprising the decidua, and RA levels ($r = -0.17$).

Discussion

In this work, we have identified “alternatively activated” M2 macrophages, delineated by the simultaneous expression of CD14 and CD163, as the major RA producers in term decidua (Figs. 2 and 4). RA plays a critical role in immune homeostasis through regulation of proinflammatory/suppressor T-cell balance (Th17, Treg lymphocytes), certain innate lymphoid cells, and maturation/differentiation of myeloid

lineage cells [4,9,41–43]. A primary source of RA production in the GIT are CD103+ dendritic cells (DC) that reside within Peyer's patches, small intestine lamina propria, and mesenteric lymph nodes [1,3,44]. RA produced from these gut-associated DC has been shown to imprint gut tropism, differentiation of IgA antibody secreting cells, and T-cell lineage determination whereby RA potentiates Treg induction and inhibits proinflammatory Th17 differentiation [3,4,42–44]. We did not observe CD103+ dendritic cells among the decidual cells isolated in our study (not shown).

Our analysis showed a close approximation to a linear regression model of decidual tissue RA concentration to the corresponding M2 cell density (Fig. 5), supporting the contention that these cells are the predominant decidual source of RA biosynthesis. Although we determined that decidual $CD10^+$ stromal cells are a lesser source of RA production on a per cell basis, we considered the possibility that, since these cells are numerically abundant, they may contribute to overall RA levels in bulk decidua tissue. This does not appear to be the case as there was no correlation between decidual RA levels and percentage of $CD10^+$ cells among the decidual cell population (Fig. 5). Our analysis indicated that the decidual lymphocyte population (both $CD3^+$ and CD3-ve cells) also showed comparatively low RALDH activity, which is consistent with that reported in peripheral blood lymphocytes [45]. Finally, we have demonstrated that only a small percentage of leukocytes in the non-lymphocyte population (<10% of the $CD45^+$ cells in the R2 gate) have phenotypes that do not express either CD14 and/or CD11c, making it unlikely that we have missed the detection of another high RA-producing cell population that significantly contribute to decidual RA levels. Together, our findings indicate that M2 macrophages are the major source of RA production in decidual tissue. However, we do not rule out the possibility of differences in RA biosynthesis by subpopulations of M2 cells that have previously been delineated among the broader population of M2 cells (e.g. those with distinct expression of CD209, CD11b, or HLA-DR) [22,36,46].

These results may provide additional insight into our understanding of maternal immune tolerance to the semi-allogeneic fetus during pregnancy and the cause of certain pregnancy complications that have been attributed to the breakdown of immune homeostasis. To this end, the increased gradient of Tregs and predominance of M2 cells in decidua compared to their counterparts in blood [23] are thought to play major roles for sustaining maternal tolerance [47,48]. Interestingly, reduction of both decidual Tregs and M2 cells has been shown to be associated with certain pregnancy-related proinflammatory conditions including, pre-term labor, recurrent spontaneous abortions, and preeclampsia [13,14,22,49–51]. Whether there is a link between the paucity of decidual M2 cells and reduced RA production in these syndromes and a loss of maternal immune tolerance will be the subject of future studies.

Declarations

ACKNOWLEDGEMENT

The authors are indebted to The Abraham J. & Phyllis Katz Foundation for their support of this project and for their continued unwavering support of numerous research, educational, and cultural programs

that have enriched our community and provided foundations for future progress in patient care.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

FUNDING SUPPORT

The Abraham J. & Phyllis Katz Foundation, the Eunice Kennedy Shriver National Institute of Child Health and Human Development/National Institutes of Health grant R01HD077260 to M. A. K. and the University of Maryland School of Pharmacy Mass Spectrometry Center (SOP1841-IQB2014).

ETHICS

Decidua basalis tissue was collected from 20 delivered placentas according to a protocol approved by the Emory Institutional Review Board (IRB00078902) as part of another study [24]. Signed informed consent was obtained from all subjects.

AUTHOR NOTE

This manuscript has been accepted for publication in the journal Reproductive Sciences.

References

1.Cassani B, Villablanca EJ, De Calisto J, Wang S, Mora JR. Vitamin A and immune regulation: role of retinoic acid in gut-associated dendritic cell education, immune protection and tolerance. *Molecular aspects of medicine.* 2012;33(1):63–76.

2.Hall JA, Grainger JR, Spencer SP, Belkaid Y. The role of retinoic acid in tolerance and immunity. *Immunity.* 2011;35(1):13–22.

3.Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song SY. Retinoic acid imprints gut-homing specificity on T cells. *Immunity.* 2004;21(4):527–538.

4.Ross AC. Vitamin A and retinoic acid in T cell-related immunity. *The American journal of clinical nutrition.* 2012;96(5):1166S–1172S.

5.Scott CL, Aumeunier AM, Mowat AM. Intestinal CD103+ dendritic cells: master regulators of tolerance? *Trends in immunology.* 2011;32(9):412–419.

6.Weiner HL, da Cunha AP, Quintana F, Wu H. Oral tolerance. *Immunological reviews.* 2011;241(1):241–259.

7.Ishikawa D, Okazawa A, Corridoni D, et al. Tregs are dysfunctional in vivo in a spontaneous murine model of Crohn's disease. *Mucosal immunology.* 2013;6(2):267–275.

8.Magnusson MK, Brynjolfsson SF, Dige A, et al. Macrophage and dendritic cell subsets in IBD: ALDH+ cells are reduced in colon tissue of patients with ulcerative colitis regardless of inflammation. *Mucosal immunology.* 2016;9(1):171–182.

9.Liu S, Diao L, Huang C, Li Y, Zeng Y, Kwak-Kim JYH. The role of decidual immune cells on human pregnancy. *Journal of reproductive immunology.* 2017;124:44–53.

10.Houser BL. Decidual macrophages and their roles at the maternal-fetal interface. *The Yale journal of biology and medicine.* 2012;85(1):105–118.

11.Lash GE, Ernerudh J. Decidual cytokines and pregnancy complications: focus on spontaneous miscarriage. *Journal of reproductive immunology.* 2015;108:83–89.

12.Quinn KH, Parast MM. Decidual regulatory T cells in placental pathology and pregnancy complications. *American journal of reproductive immunology.* 2013;69(6):533–538.

13.Quinn KH, Lacoursiere DY, Cui L, Bui J, Parast MM. The unique pathophysiology of early-onset severe preeclampsia: role of decidual T regulatory cells. *Journal of reproductive immunology.* 2011;91(1–2):76–82.

14.Rahimzadeh M, Norouzian M, Arabpour F, Naderi N. Regulatory T-cells and preeclampsia: an overview of literature. *Expert review of clinical immunology.* 2016;12(2):209–227.

15.Laws MJ, Taylor RN, Sidell N, et al. Gap junction communication between uterine stromal cells plays a critical role in pregnancy-associated neovascularization and embryo survival. *Development.* 2008;135(15):2659–2668.

16.Tanmahasamut P, Sidell N. Up-regulation of gap junctional intercellular communication and connexin43 expression by retinoic acid in human endometrial stromal cells. *The Journal of clinical endocrinology and metabolism.* 2005;90(7):4151–4156.

17.Wu J, Taylor RN, Sidell N. Retinoic acid regulates gap junction intercellular communication in human endometrial stromal cells through modulation of the phosphorylation status of connexin 43. *Journal of cellular physiology.* 2013;228(4):903–910.

18.Fukunaka K, Saito T, Wataba K, Ashihara K, Ito E, Kudo R. Changes in expression and subcellular localization of nuclear retinoic acid receptors in human endometrial epithelium during the menstrual cycle. *Molecular human reproduction.* 2001;7(5):437–446.

19.Ozaki R, Kuroda K, Ikemoto Y, et al. Reprogramming of the retinoic acid pathway in decidualizing human endometrial stromal cells. *PloS one.* 2017;12(3):e0173035.

20.Vermot J, Fraulob V, Dolle P, Niederreither K. Expression of enzymes synthesizing (aldehyde dehydrogenase 1 and reinaldehyde dehydrogenase 2) and metabolizaing (Cyp26) retinoic acid in the mouse female reproductive system. *Endocrinology.* 2000;141(10):3638–3645.

21.Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. *The Journal of pathology.* 2013;229(2):176–185.

22.Schonkeren D, van der Hoorn ML, Khedoe P, et al. Differential distribution and phenotype of decidual macrophages in preeclamptic versus control pregnancies. *The American journal of pathology.* 2011;178(2):709–717.

23.Svensson-Arvelund J, Mehta RB, Lindau R, et al. The human fetal placenta promotes tolerance against the semiallogeneic fetus by inducing regulatory T cells and homeostatic M2 macrophages. *Journal of immunology.* 2015;194(4):1534–1544.

24.Deepak V S, Badell ML, Sidell N, & Rajakumar A. Retinoic acid downregulates sflt1 in decidual stromal cells independent of decidualization. Implications to preeclampsia. *Pregnancy hypertension.* 2018;13S1:S28.

25.Cottrell HN, Wu J, Rimawi BH, et al. Human endometrial stromal cell plasticity: Reversible sFlt1 expression negatively coincides with decidualization. *Hypertension in pregnancy.* 2017;36(2):204–211.

26.Olivares EG, Montes MJ, Oliver C, Galindo JA, Ruiz C. Cultured human decidual stromal cells express B7–1 (CD80) and B7–2 (CD86) and stimulate allogeneic T cells. *Biology of reproduction.* 1997;57(3):609–615.

27.Oliver C, Cowdrey N, Abadia-Molina AC, Olivares EG. Antigen phenotype of cultured decidual stromal cells of human term decidua. *Journal of reproductive immunology.* 1999;45(1):19–30.

28.Yokota A, Takeuchi H, Maeda N, et al. GM-CSF and IL–4 synergistically trigger dendritic cells to acquire retinoic acid-producing capacity. *International immunology.* 2009;21(4):361–377.

29.Kane MA, Napoli JL. Quantification of endogenous retinoids. *Methods in molecular biology.* 2010;652:1–54.

30.Jones JW, Pierzchalski K, Yu J, Kane MA. Use of fast HPLC multiple reaction monitoring cubed for endogenous retinoic acid quantification in complex matrices. *Analytical chemistry.* 2015;87(6):3222–3230.

31.Kane MA, Folias AE, Napoli JL. HPLC/UV quantitation of retinal, retinol, and retinyl esters in serum and tissues. *Analytical biochemistry.* 2008;378(1):71–79.

32.Carbonari M, Tedesco T, Fiorilli M. A unified procedure for conservative (morphology) and integral (DNA and immunophenotype) cell staining for flow cytometry. *Cytometry.* 2001;44(2):120–125.

33.Medeiros LT, Peracoli JC, Bannwart-Castro CF, et al. Monocytes from pregnant women with pre-eclampsia are polarized to a M1 phenotype. *American journal of reproductive immunology.* 2014;72(1):5–13.

34.Tang Y, Hua SC, Qin GX, Xu LJ, Jiang YF. Different subsets of macrophages in patients with new onset tuberculous pleural effusion. *PloS one.* 2014;9(2):e88343.

35.Hsu P, Nanan RK. Innate and adaptive immune interactions at the fetal-maternal interface in healthy human pregnancy and pre-eclampsia. *Frontiers in immunology.* 2014;5:125.

36.Rieger L, Segerer S, Bernar T, et al. Specific subsets of immune cells in human decidua differ between normal pregnancy and preeclampsia—a prospective observational study. *Reproductive biology and endocrinology: RB&E.* 2009;7:132.

37.Guo Y, Brown C, Ortiz C, Noelle RJ. Leukocyte homing, fate, and function are controlled by retinoic acid. *Physiological reviews.* 2015;95(1):125–148.

38.Villablanca EJ, Wang S, de Calisto J, et al. MyD88 and retinoic acid signaling pathways interact to modulate gastrointestinal activities of dendritic cells. *Gastroenterology.* 2011;141(1):176–185.

39.Best MW, Wu J, Pauli SA, et al. A role for retinoids in human oocyte fertilization: regulation of connexin 43 by retinoic acid in cumulus granulosa cells. *Molecular human reproduction.* 2015;21(6):527–534.

40.Pierzchalski K, Taylor RN, Nezhat C, et al. Retinoic acid biosynthesis is impaired in human and murine endometriosis. *Biology of reproduction.* 2014;91(4):84.

41.Larange A, Cheroutre H. Retinoic Acid and Retinoic Acid Receptors as Pleiotropic Modulators of the Immune System. *Annual review of immunology.* 2016;34:369–394.

42.Mucida D, Park Y, Kim G, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science.* 2007;317(5835):256–260.

43.Sun CM, Hall JA, Blank RB, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *The Journal of experimental medicine.* 2007;204(8):1775–1785.

44.Iwata M. Retinoic acid production by intestinal dendritic cells and its role in T-cell trafficking. *Seminars in immunology.* 2009;21(1):8–13.

45.Xi J, Yang Z. Expression of RALDHs (ALDH1As) and CYP26s in human tissues and during the neural differentiation of P19 embryonal carcinoma stem cell. *Gene expression patterns: GEP.* 2008;8(6):438–

442.

46.Faas MM, Spaans F, De Vos P. Monocytes and macrophages in pregnancy and pre-eclampsia. *Frontiers in immunology.* 2014;5:298.

47.Aluvihare VR, Kallikourdis M, Betz AG. Tolerance, suppression and the fetal allograft. *Journal of molecular medicine.* 2005;83(2):88–96.

48.Santner-Nanan B, Peek MJ, Khanam R, et al. Systemic increase in the ratio between Foxp3+ and IL–17-producing CD4+ T cells in healthy pregnancy but not in preeclampsia. *Journal of immunology.* 2009;183(11):7023–7030.

49.Nagamatsu T, Schust DJ. The immunomodulatory roles of macrophages at the maternal-fetal interface. *Reproductive sciences.* 2010;17(3):209–218.

50.Wang S, Sun F, Han M, et al. Trophoblast-derived hyaluronan promotes the regulatory phenotype of decidual macrophages. *Reproduction.* 2019;157(2):189–198.

51.Xu Y, Romero R, Miller D, et al. An M1-like Macrophage Polarization in Decidual Tissue during Spontaneous Preterm Labor That Is Attenuated by Rosiglitazone Treatment. *Journal of immunology.* 2016;196(6):2476–2491.

Figures

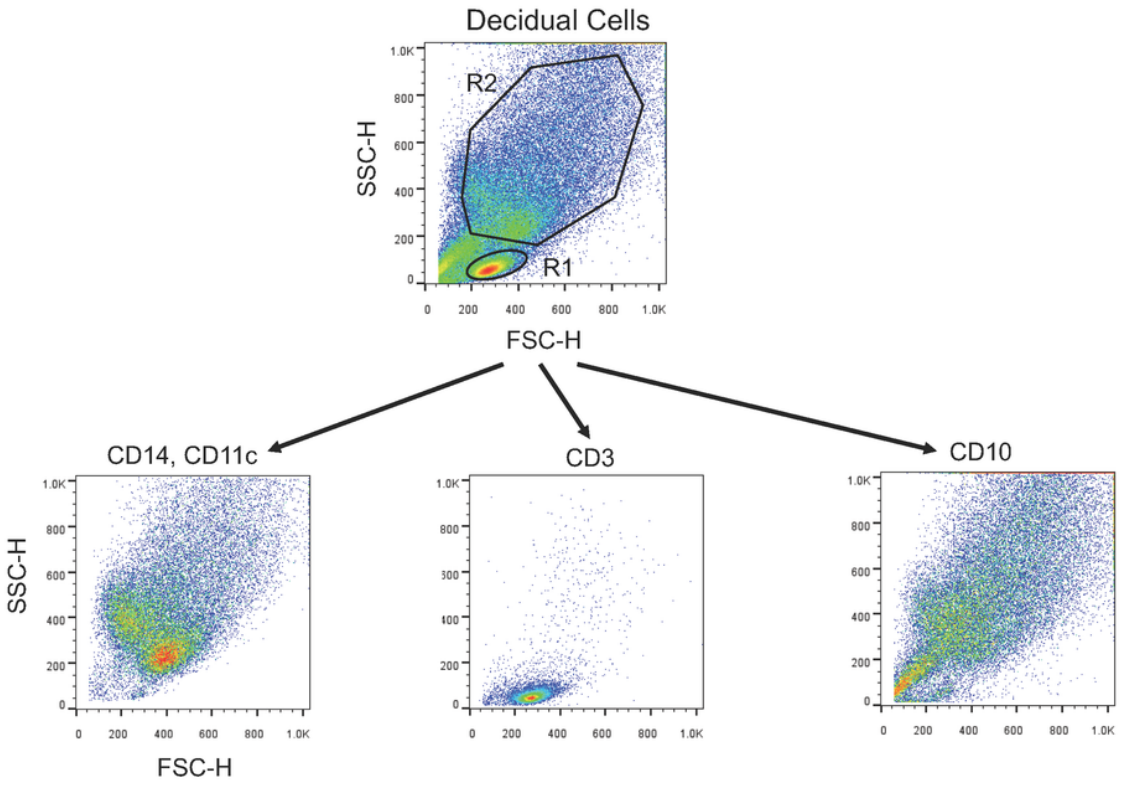

Fig. 1

Figure 1

Identification and scatter characteristics of decidual cell populations obtained by collagenase digestion and stained with specific fluorescence-conjugated antibodies. Representative scatter plots of decidual cells stained with antibodies specific for the indicated CD subpopulations are shown in the lower panel. Subsequently, CD3+ lymphocytes and CD14+/CD11c+ cells were analyzed in the “R1” and “R2” gates, respectively, as drawn on the scatter plot in the upper panel.

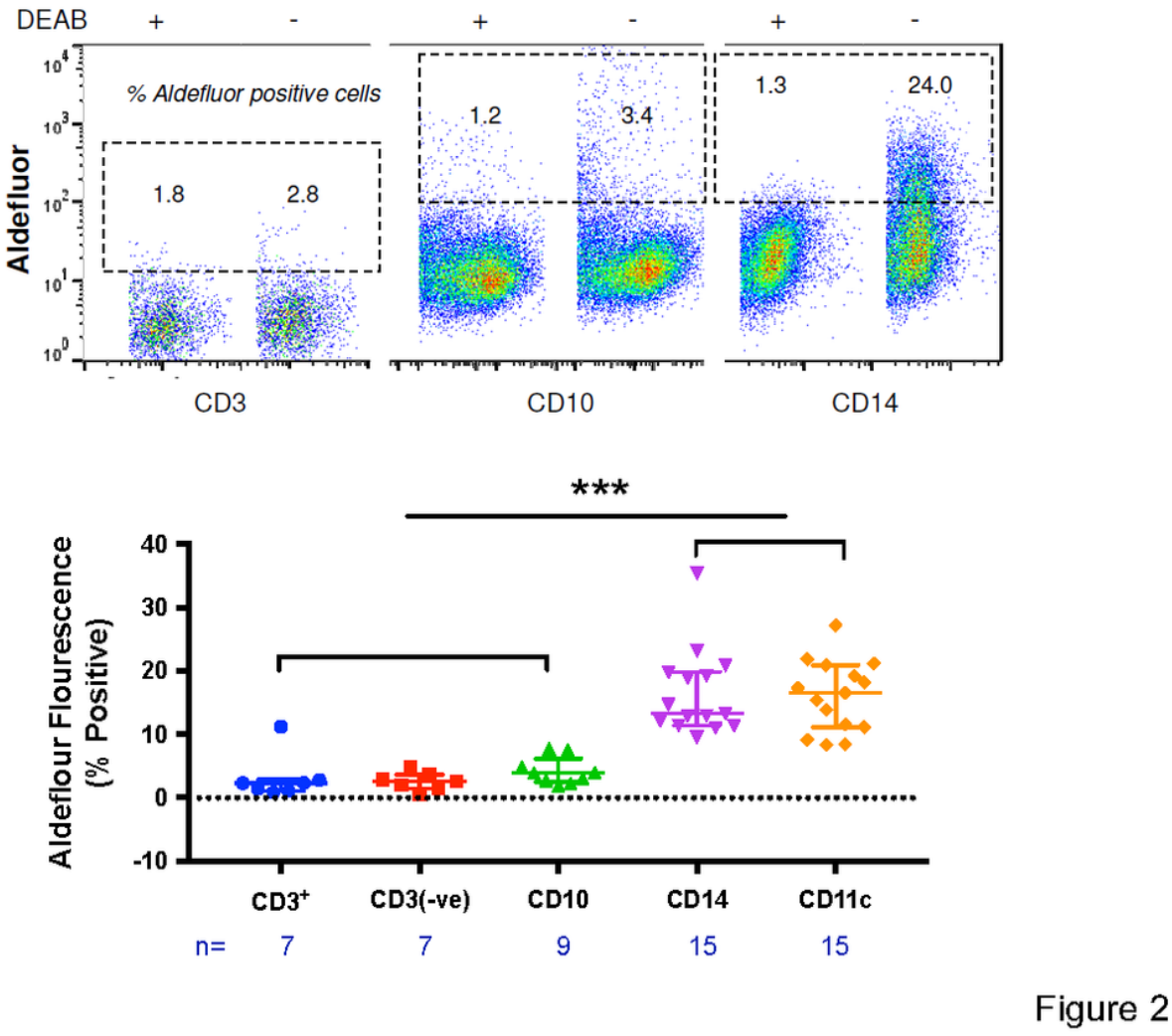

Figure 2

Figure 2

RALDH activity (Aldefluor fluorescence) in subpopulations of decidual cells. RALDH activity of dispersed mononuclear cells isolated from term placenta decidua was determined using the Aldefluor assay combined with flow cytometry for evaluating CD marker expression. DEAB-treated cells were used to evaluate background fluorescence in order to set negative control levels as demonstrated in the representative experiment showing aldefluor florescence of CD3, CD10, and CD14 subpopulations (upper panel). The scatter plot in the lower panel shows the median and interquartile range of percent positive fluorescent cells expressed by the indicated CD lineage. The number of data sets (placentas) utilized for analysis of the subpopulations (n value) is indicated. ***P< 0.001

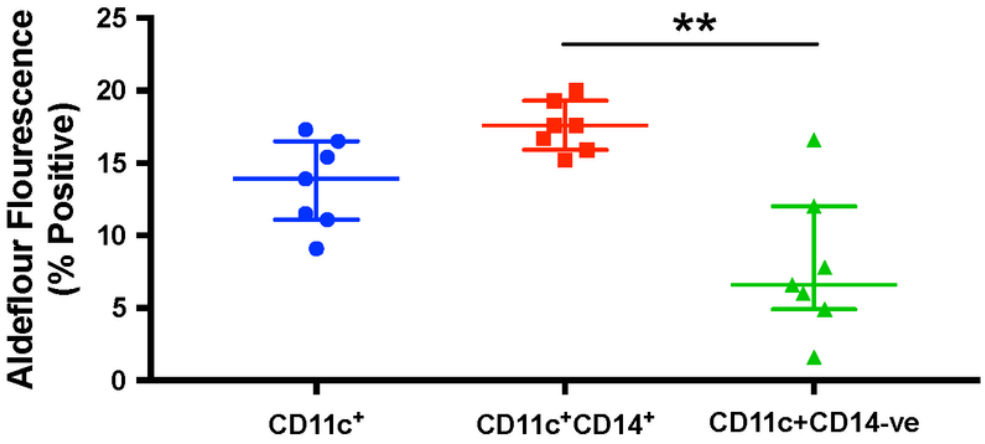

Figure 3

Figure 3

CD11c+ cells that co-express CD14 show higher levels of RALDH activity than those that are CD14-ve. Dispersed decidual cells were gated according to their co-expression of CD11c and CD14 by multicolor flow cytometry and analyzed for RALDH activity by the Aldefluor assay. The scatter plot shows the median and interquartile range of percent positive fluorescent cells (n = 7 patient samples). **P< 0.005

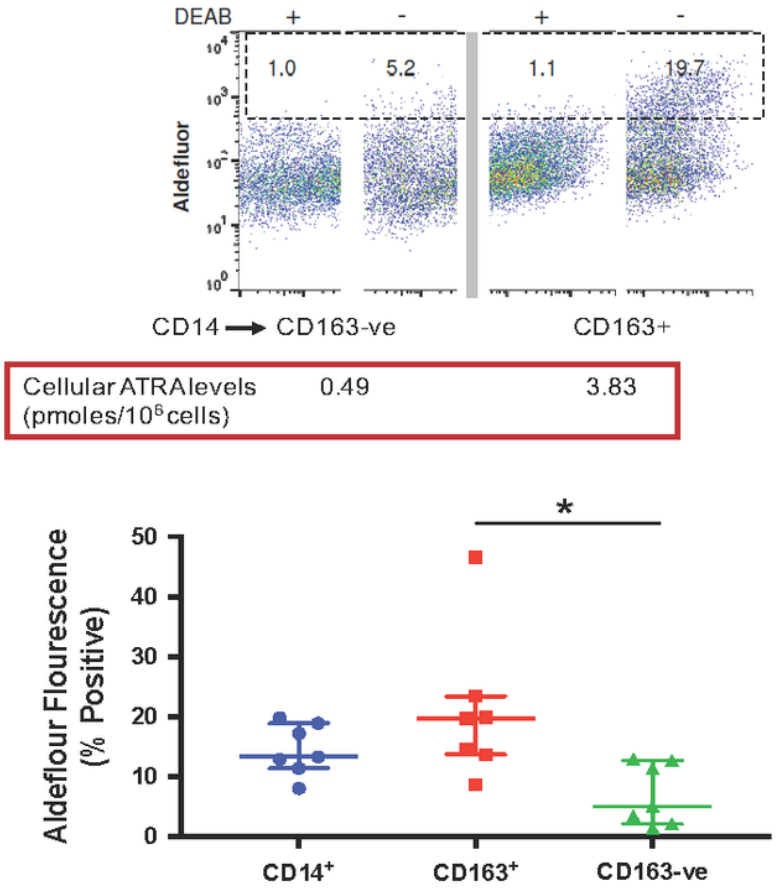

Figure 4

Figure 4

The RA synthesizing capacity of CD14+ cells is dependent on their polarization state. Classically activated M1 (CD14+CD163-ve) and alternatively activated M2 (CD14+CD163+) cells were analyzed for RALDH activity by the Aldefluor assay of dispersed decidual cells gated according to their co-expression of CD14 and CD163. In some experiments, cells were purified into M1 and M2 subpopulations by fluorescent cell sorting. The ability of the sorted cell populations to synthesize RA was assessed by LC-MS/MS analysis following addition of 2 μM retinol for 6 hr under serum-free conditions. The upper panel shows a representative experiment (of n = 3 patient samples) which validated the relationship between RALDH activity and the RA synthesizing capacity of the M2 versus M1 populations. The scatter plot in the lower panel shows the median and interquartile range of percent positive fluorescent cells (n = 7 patient samples) and indicates that RALDH activity in M2 cells is greater than that in M1. *P < 0.01.

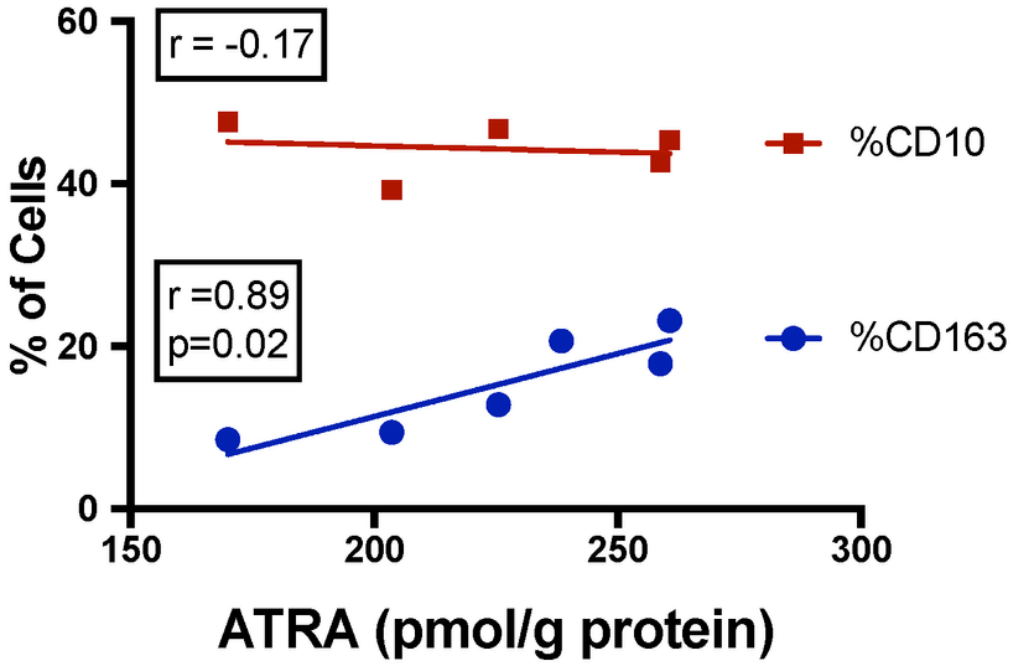

Figure 5

Figure 5

Comparison of RA levels in decidual tissues with percentage of M2 (CD163+) or stromal (CD10+) cells. Endogenous RA concentration (pmol/g protein) in whole decidua tissue was assessed by LC-MS/MS. Percentage of cell populations in each sample with respect to total cell number was determined by multicolor flow cytometry. RA concentrations in the decidua samples are highly correlated with the percentage of M2 cells (r = 0.89) but not with CD10+ stromal cells (r = -0.17) in the specimens (Pearson's correlation analysis). Solid line equals regression line.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ReproductiveSciencesDecisionLetter.png