Supporting Information

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SI Materials and Methods

Isolation and culture of CD34$^+$ cells and decidual stromal cells. To obtain CD34$^+$ cell precursors, we first depleted CD56$^+$ NK cells by negative beads selection (Invitrogen Dynal). dCD34$^+$ cells were then obtained using CD34 MultiSort kit (Miltenyi). We first removed VEGF-R2$^+$ cell fraction that includes CD34$^+$ cells characterized by high FSC and SSC parameters. The resulting cells (highly enriched in mononuclear cells with low FSC and low SSC parameters) were further fractionated on the basis of the bright expression of CD34 antigen. CD34$^+$ cell numbers obtained ranged between 1.6×10^4 and 9.6×10^4 g of decidual tissue processed. We purified by positive selection CB-CD34$^+$ cells with CD34 Progenitor cell Isolation kit (Miltenyi). Cells were routinely >98% pure. We plated 25×10^4 CD34$^+$ cells in 24-well plates in RPMI-1640 medium supplemented with 5% FCS, 10% human AB serum (BioWhittaker/Cambrex), and the following cytokines: SCF, FMS-Like Flt3-L, IL-7, IL-15 (Peprotech), IL-21 (BioSource/Invitrogen), 20 ng/ml each; cells were also cultured with GM-CSF (20 ng/ml). To obtain dSC, fresh decidual cell suspensions were cultured in 10% FCS-RPMI medium, and after 24 h nonadherent cells were removed. Fresh CD3$^+$ T, CD20$^+$ B, and CD3-CD56$^+$ NK lymphocytes, isolated from decidua, CB and PB were purified by cell sorting.

mAbs. We used a large panel of mAbs against different molecules and surface receptors. The following specific mAbs were produced in our laboratory or were provided by D. Pende, A. Moretta, and A. Poggi: KD1 (CD16, IgG2a), c218 (CD56, IgG1), GPR165 (CD56, IgG2a), 7A6 (NKp30, IgG1), BAT221 (NKG2D, IgG1), BAB281 (NKp46, IgG1), Z231 (NKp44, IgG1), AZ20 (NKp44, IgG1), PP35 (2B4, IgG1), Z270 (NKG2A, IgG1), 11PB6 (CD158a, IgG1), GL183 (CD158b, IgG1), Z27 (CD158e, IgG1), 191B8 (CD161, IgG2a), JT90 (LFA-1, IgG2a), and T96H6 (LFA-1, IgG1). We purchased the following specific mAbs from BD-Pharmingen: CD7 (IgG1), CD45-RA (IgG1), CD45 (IgG1), CD33 (IgG1), CD122-PE (IgG2a), CD127-PE (IgG1), CD34 (IgG1) and CD20 (IgG2b); CD34-APC was purchased from Biolegend; CD56-PC7 from Coulter-Immunotech; CD161-APC, CD133 (IgG1), CD117-PE, CD3-FITC, and VEGFR2-APC from Miltenyi; and mIL-15 and IL-15Rα from R&D Systems. Secondary conjugated-specific mAbs were from Invitrogen or Southern Biotech.

Immunohistochemical analysis. Decidual samples were formalin fixed and paraffin embedded. We deparaffinized and subsequently exposed the 7-m sections to 0.3% hydrogen peroxide–methanol solution to quench the endogenous peroxidase activity. We incubated sections for 30 min at room temperature with CD34 (Dako), CD45, and CD56 specific mAbs (Abcam) (10 g/ml). We added HRP-conjugated goat anti-human anti-sera (Dako-Envision) and 3-amino-9-ethylcarbazole (Novocastra). We counterstained specimens in Mayer's haemalum and coverslipped them. Negative control was an isotype-matched mAb. Two independent observers interpreted results under light microscope.

Cytolytic assay. We analyzed cell cytotoxicity in a 4-h 51Cr–release assay against the FcRγ+P815 mastocytoma murine cell line as previously described (1). We performed experiments in duplicates; data are expressed as percentage of target cell lysis. The E/T ratio was 5/1.

Analysis of cytokine release. At day 15 we collected supernatants derived from culture of dCD34 cells and analyzed them for the presence of IL-8 and IL-22 by Flow-Cytomix kit immunoassay (Bender Med System) according to the manufacturer's instructions. Data were analyzed by BenderMed System Flow-Cytomix Software.

Real-Time RT-PCR Analysis. Total RNA from purifed fresh CD34, NK, T, and B cells from decidua, CB, and PB were analyzed by using an RNAeasy Micro Kit (Qiagen). Oligo (dT)-primed cDNA was prepared by standard technique using Transcriptor (Roche). Relative quantitation of gene expression was performed in real-time RT-PCR using GAPDH as housekeeping gene. RORC PCR products were run on a 0.8% agarose gel and visualized by ethidium bromide staining. Relative quantitation of gene expression was performed in real-time RT-PCR with Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen) and GAPDH as housekeeping gene. Amplifications were performed with a Mastercycler ep realplex (Eppendorf) in a 20-μL final volume, using primers at 300 nM for 40 cycles (15 s at 95 °C, 30 s at 60 °C). A dissociation curve was carried out at the end of 40 cycles to confirm specificity of amplification. The following primers were used: GAPDH forward, 5′-GAAGGTGAAGGTCGGAGT-3′; GAPDH reverse, 5′-CATGGGTGGAATCATATTGGAA-3′; E4BP4 forward, 5′-CCAAGGGCCCCATCCATTC-3′, E4BP4 reverse, 5′-GATGCCAGTGCTCCGATTTG-3′; Id2 forward, 5′-GCAAAACCCCTGTGGACG-3′; Id2 reverse, 5′-CAGAAGCCTGCAAGGACAG-3′; RORC forward, 5′-TTTTCCGAGGATGAGATTGC-3′; and RORC reverse, 5′-CTTTCCACATGCTGGCTACA-3′. Relative expression of each transcript was obtained using the $\Delta\Delta C_T$ method.

1. Sivori S, et al. (2004) CpG and double-stranded RNA trigger human NK cells by Tolllike receptors: Induction of cytokine release and cytotoxicity against tumors and dendritic cells. *Proc Natl Acad Sci USA* 101:10116–10121.

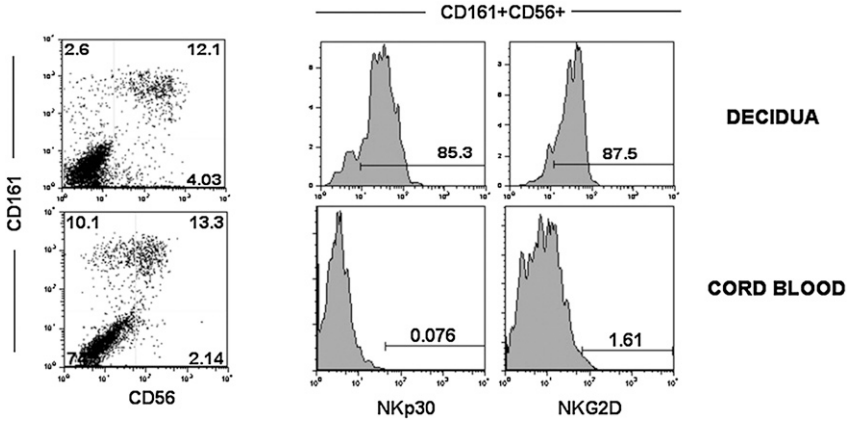

Fig. S1. Comparative analysis of the surface expression of NKp30 and NKG2D in dCD34$^+$ and CBCD34$^+$ cells cultured in vitro for 15 d. Purified CD34$^+$ cells isolated from decidua or CB cells were cultured for 15 d in the presence of cytokine mix and analyzed for the surface expression of the activating receptors NKp30 and NKG2D.

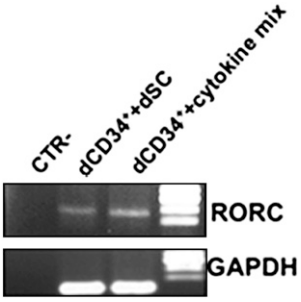

Fig. S2. Analysis of RORC expression in cultured dD34$^+$ cells. RT-PCR analysis of RORC and GAPDH in dCD34$^+$ cells cultured for 15 d in the presence of dSCs or cytokine mix.

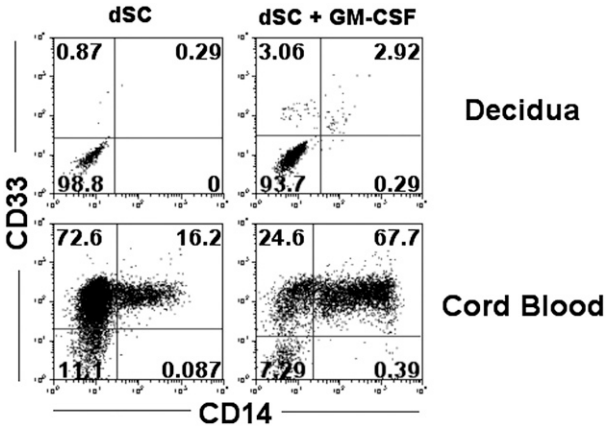

Fig. S3. Human dCD34$^+$ cells do not differentiate into myeloid cells. Decidual or CB CD34$^+$ cells were cultured for 10 d together with dSCs in the presence or in the absence of GM-CSF. Cells were analyzed by flow cytometry for the surface expression of the myeloid markers CD33 and CD14.