Supplementary Information

Supplementary Methods

RT- PCR, PCR

Total RNA (1μg) was converted into cDNA using the reverse transcription system kit (Promega), in a reaction containing: 500ng of oligonucleotide A ($T_{15}V$), 1mM deoxynucleotriphosphates, 5mM $MgCl_2$, 10mM Tris pH8.8, 50mM KCl, 0.1%Triton X-100, 18 units of Avian Myeloblastosis virus reverse transcriptase and 10 units of RNasin ribonuclease inhibitor. Each reaction was incubated at 42°C for two hours, then cDNA were diluted to 80μl adding to the reaction 60μl of deionized H_2O. Polymerase chain reactions were performed using 5μl of cDNA as template and then adding: 500nM each of forward and reverse primer, 0.2mM dNTPs, 2mM $MgCl_2$, 10mM Tris pH8.8, 50mM KCl, 0.1%Triton X-100 and 1 unit of AmpliTaq Gold (Applied Biosystems). The following primers were used for the Vγ2 (Vγ9 according to the IMGT nomenclature) chain: oligo Vγ2 (5'ATC AAC GCT GGC AGT CC 3'); oligo Cγ1 (5'GTT GCT CTT CTT TTC TTG CC 3'). For the Vδ1 chain we used: oligo Vδ1 (5' TTA ACC ATT TCA GCC TTA CAG C 3') and oligo Cδ-1 (5' TGG CAG TCA AGA GAA AAT TG 3'). PCR were run with the following profile: denaturation: 1 min at 94°C; 5 min at 68°C; 45 cycles (45s at 94°C, 1 min at 60°C, 1 min at 72°C); extension: 10min at 72°C. PCR products were separated on 1.5% agarose/Tris-acetate-EDTA buffer gels containing 0.5μg/ml ethidium bromide (Sigma).

Run-off reaction.

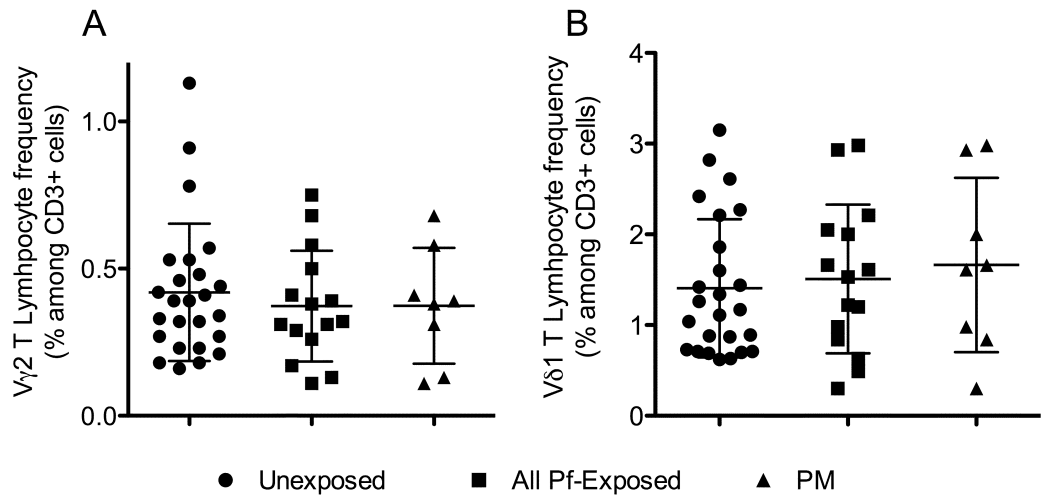
Primer extension reactions were performed as described [37]. Each reaction contained 2 μl of PCR product, 3 mM $MgCl_2$, 0,2 mM dNTP, 0.1 mM of 6-carboxyfluorescein (6-FAM) labeled primer (Cg-6:

5'-6-FAM–AAT AGT GGG CTT GGG GGA AAC-3'; Jg1.2: 5'-6-FAM–; Cd:5'-6-FAM–ACG GAT GGT TTG GTA TGA GG-3'), approximately 0.2 units of Taq DNA polymerase (Promega), 10 mM Tris pH8.8, and 50 mM KCl. Run-off products (4 μl) were diluted with deionized formamide (6 μl), and 0.7 μl of GeneScan-500 ROX size standard (Applied Biosystems) were added to each sample. After a denaturation step (5 min at 95°C followed by immediate quenching on ice) products were loaded on a 3130 genetic analyzer (Applied Biosystems) and run on a performance-optimized polymer (POP-7).

Sequencing

PCR products for the Vγ2 chain were purified by gel extraction, using QIAquick gel extraction kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. Purified products were denatured (1 minute at 94°C), then incubated for 30 minutes at 72°C with 2mM $MgCl_2$, 0.2mM dATPs, and 2.5 units of Amplitaq gold (Promega, Madison, WI), then ligated into a pCR2.1 vector (Invitrogen, Carlsbad, CA). Ligated vectors were transfected into TOP 10F' competent cells (TA cloning kit, Invitrogen, Carlsbad, CA), and bacterial colonies representing a library of Vγ2 chain sequences were grown overnight on agar plates containing 50μg/ml ampicillin, 500 μM IPTG and 80 μg/ml X-Gal (Promega, Madison, WI). Colonies containing recombinant plasmids were cultured overnight in LB media and bacterial suspensions were used as template to amplify the Vγ2 chain inserted in the plasmid. M13 polymerase chain reactions were performed using: 2 μl of bacterial suspension as template, 100 nM of M13 forward (5' GTA AAA CGA CGG CCA G 3') and M13 reverse primer (5' CAG GAA ACA GCT ATG AC 3'), 0.2 mM dNTPs, 2 mM $MgCl_2$, 10 mM Tris pH 8.8, 50 mM KCl, 0.1%Triton X-100 and 1 unit of AmpliTaq Gold (Promega, Madison, WI). PCR were run with the following profile: denaturation: 1 min at 94°C; 25 cycles (45 s at 94°C, 1 min at 60°C, 1 min at 72°C); extension: 10 min at 72°C. PCR products were purified by size exclusion, after running through a bed of Sephacryl S400

(GE Healthcare, Uppsala, Sweden) packed (200 µl/well) into 96 well MultiScreen HTS filtration plates (Millipore, Billerica, MA). Sequencing reactions were performed with a Big Dye v3.1 fluorescent sequencing kit (Applied Biosystems, Foster City, CA), and M13F or M13R oligonucleotide primers for each sample. Sequencing reactions were run with the following profile: denaturation: 1 min at 94°C; 25 cycles (30 s at 96°C, 20 s at 50°C, 4 min at 60°C). Sequences were loaded on an automated sequencer ABI3700 and analyzed using Sequencher and MacClade softwares.



Supplementary Figure S1

Supplementary Figure 1. Prenatal exposure to *Pf* did not affect the levels of γδ T cell populations in cord blood. The frequency of A) Vγ2+ lymphocytes (Vγ9 according to IMGT nomenclature) and B) Vδ1+ lymphocytes was analyzed by flow-cytometry in freshly isolated CBMC and reported as fraction of CD3+ cells. The scatter plots show individual values, means and standard deviations for Unexposed, All *Pf*-exposed and PM neonates.

Supplementary Table 1. Vγ2Vδ2 T cell phenotype 14 days after ALN stimulation.

	Unexposed (N=16)	Pf-Exposed (N=15)
CD56	39.3±14.0 (20.6-68)	34.1±18.9 (7.2-80)
NKG2A	19.2±12.2 (5.5-51.4)	15.3±7.8 (4.3-33.3)
NKG2D	73.5±14.6 (50.8-92.7)	66.2±18.1 (18-91.2)
CD25[a]	220.2±137.3 (50.1-445)	240.2±190.9 (52.9-607)
CD45RA-CD27+	70.5+17.8 (27.1-89.8)	69.8±15.3 (30-89.2)
CD45RA-CD27-	18.1±14.4 (1.6-53.4)	19.5±14.4 (1.4-62.7)

Means ± Standard deviations (range) are shown for each group. Values (with the exception of the CD25 row) represent the percentages of Vδ2 T cells displaying a certain phenotype.

[a] Mean Fluorescence Intensity