

Supplementary figure 1: Anatomy of human placenta (adapted from *Maltepe et al. 2010* and *Robbins et al. 2009*)

The placenta is a chimeric organ at the interface between fetal and maternal blood. **a.** The human placenta form a villous tree bathed in maternal blood and is composed of differentiated fetal cells from a common origin: the fetal trophoblasts. **b.** Most villi of this villous tree are floating freely in the maternal blood (FV). Some villi (AV) anchor the placenta in the maternal endometrium by invading the uterine lining. At the tip of these AV, fetal trophoblasts, called Extra Villous Trophoblasts (EVT), invade and modify the endometrium and form the decidua. **c.** Fetal blood capillaries are surrounded by a stroma made of fibroblast and by trophoblasts (vCTB); at the interface with maternal blood, trophoblasts differentiate in a multinucleated syncytium (SYN).



Figure 1:

0

0 10²

10

GFP

Characterization of the infection of human PBSC with a GFP expressing strain of *L. monocytogenes*: **a.** Six leukocyte subpopulations were distinguished: (1) monocytes CD14^{high} CD16^{low}, (2) monocytes CD14^{high} CD16^{high}, (3) neutrophils (CD14^{low} CD16^{low}), (4) NK cells (CD14^{neg} CD16^{high}), (5) monocyte bone marrow precursor (CD14^{neg} CD16^{neg} CD34^{high}) (6) the remainders were mainly lymphocytes. **b.** Infected leukocyte subpopulations of human PBSCs analyzed by FACS and separated depending on the expression of CD34 and GFP. **c.** Average proportion of GFP+ cells per subpopulation. Averages were established on 6 independent experiments. Error bars represent the SEM.

102

n

103

GFP

104

105



5

CD34^{high}

6

CD14^{neg}

CD16^{neg}



Supplementary figure 2:

Characterization of the infection of human PBSC by a GFP expressing strain of *L. monocytogenes*: **a.** The six leukocyte subpopulations characterization over time after infection. The population (2) of monocytes $CD14^{high}CD16^{high}$ disappears progressively over time. **b.** Specific sorting of the two monocyte populations (CD14^{high}) separated on the size and scatter (FSC, SSC subset) and on the expression of CD14 and CD16 before infection. Two populations (A and B) are defined corresponding to monocytes $CD14^{high}CD16^{low}$ (A and 60% of B) and $CD14^{high}CD16^{high}$ (40% of B). **c.** After infection the ratio A:B is stable but no $CD14^{high}CD16^{high}$ cells are detectable in the B population.





d



Figure 2:

Intracellular growth of L. monocytogenes in primary human monocytes and neutrophils compared to the monocytic cell line U937. a. $(CD14^{high}CD16^{low})$ monocytes and CD14^{high}CD16^{high}) and neutrophils (CD14^{low}CD16^{low}) were selected from PBSC by sorting. Subpopulations were cell selected depending on the size and scatter (FSC, SSC subset) and depending on the expression of CD14 and CD16 markers. b. Relative entry of bacteria into cells. The total CFU number was corrected by the cell density (see method section). c. Relative bacterial intracellular growth over the time into leukocytes subpopulation. Error bars represent the SEM.

d. Leukocytes were infected with a GFP expressing strain of *Listeria monocytogenes* and stained with DAPI. Pictures are representative of infected cells 8 hours after infection. Bars represent 10μ m.



Figure 3:

Characterization of the CD45^{high} decidual leukocytes by FACS after infection with a GFP expressing strain of *L. monocytogenes*. Separation of subpopulations using the expression of CD14 and CD56 markers and then depending on the expression of the GFP. **a.** Two subpopulations were distinguished: decidual macrophages (CD14^{low}CD56^{neg}), and decidual NK cells (CD14^{neg}CD56^{high}). About 40% of the CD14^{low} macrophages were GFP^{high} and 20% of the CD56^{high} NK cells were GFP^{high}. **b.** Proportion of GFP positive cells was quantified at 2hours p.i. in both cell types. Averages were established on 3 independent experiments. Error bars represent the SEM.



Figure 4:

a-d: Intracellular growth of *L. monocytogenes* in primary decidual macrophages and NK cells. Cells were separated depending on the expression of CD14 or CD56 (see methods section), infected with the GFP expressing strain of *L. monocytogenes* and stained with DAPI. Intracellular growth was determined by visual counting. The proportion of infected cells over time as well as the number of bacteria per cell were determined for (**a**) U937, (**b**) decidual macrophages (CD14^{low}) and (**c**) decidual NK cells (CD56^{high}). Averages were established on at least 3 independent experiments; error bars represent the SEM for the proportion of infected cells independently of the number of bacteria per cell. Pictures in panels **d** are representative of infection at 24hrs p.i.. Bars represent 10µm. **e.** Decidual leukocytes were purified depending of the expression of CD45 and infected with the GFP expressing strain. After 2, 5 or 8 hours of infection, cells were stained with AnnexinV-APC (apoptotic) and 7-AAD (dead cells) and expression of these markers was analyzed by FACS. Proportion of apoptotic or dead cells are presented for decidual macrophages and decidual NK cells. Averages were established on 3 independent experiments.



Figure 5:

Analysis of bacterial invasion and intracellular growth in CD45^{neg} decidual fibroblasts. Decidual fibroblasts were purified based on their adhesive properties (see methods section) and infection was compared to the BeWo cell line. **a.** Fibroblasts were characterized by immunofluorescence as CD45^{neg} (leukocyte), desmin^{neg} (differentiated decidual cells) and CK7^{neg} (trophoblasts) cells. **b.** Initial entry determined by CFU counting of bacteria in decidual fibroblasts or BeWo cells infected with the WT strain and with a mutant that doesn't express InIA nor InIB. Error bars represent the SEM. **c.** Relative intracellular growths of bacteria in decidual fibroblasts or BeWo cells. Averages were established on 3 independent experiments; error bar represent SEM. Picture in panel **d** is representative of infection at 24hrs p.i.. Bars represent 50 μ m (**a** and **d**).





Fig. 6.

Infection of placental explants with previously infected U937, peripheral blood derived monocytes (CD14^{high}CD16^{low} and CD14^{high}CD16^{high}) and neutrophils (CD14^{low}CD16^{low}) and decidual macrophages (CD14^{high}) and NK cells (CD56^{high}). Leukocytes were stained with a green cell tracker (see methods) and then infected with the GFP expressing strain of *Listeria monocytogenes* for 1 hour. **a.** Human placental explants were then infected with these different cells in presence of Gentamicin for 24 hours. After infection, explants were fixed with PFA and stained with DAPI (blue) and Phalloidin (red). The actin structure allowed differentiating EVT from other structure. Pictures illustrate green stained U937 (arrowheads) in contact with placental explants. Bars represent 100μ m. **b**. In each case, the number of green leukocytes as well as their location was enumerated per unit of surface. Averages were established on at least 3 independent experiments. Error bars are SEM. *p<0.05; **p<0.005.

b

Figure 7:

Transfert of *L. monocytogenes* from infected leukocytes to placental explants. Infection of placental explants with previously stained and infected U937, peripheral blood derived monocytes (CD14^{high}CD16^{low} and CD14^{high}CD16^{high}) and neutrophils (CD14^{low}CD16^{low}) and decidual macrophages (CD14^{high}) and NK cells (CD56^{high}). **a.** After infection, explants were frozen, sliced, fixed and stained with DAPI and an anti-HCG antibody to characterize the Syncytium. Pictures illustrate green stained blood monocyte CD14^{high} (arrow) in contact with placental explants at EVTs and bacteria infecting these EVTs from the monocytes. Bars represent 50μ m (left) and 10μ m (right). In each case, the number of bacteria was enumerated (**b**) and their location was deciphered(**c**). Averages were established on at least 3 independent experiments. Error bars are SEM.