Maternal-to-Fetal Leukocyte Trafficking in Fetal Maldevelopment.

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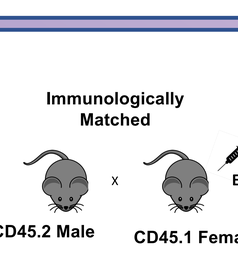
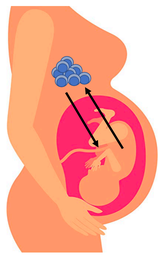
Introduction

Neural tube defects (NTDs) are severe birth defects that originate during embryonic development when the neural tube fails to close completely. They represent a major public health concern affecting 1 in 1000 pregnancies with the prospect of severe lifelong disease[1]. NTDs are major malformations, occurring during the fourth week of human gestation resulting in anencephaly or spina bifida of the central nervous system where the canal of the brain or the spinal cord is exposed to the amniotic fluid. The unprotected fetal neural tissue undergoes progressive damage with advancing gestational age, due to chemical and mechanical factors related to exposure to the intrauterine environment[2,3]. As a result, there is severely impaired brain and spinal axonal pathways. The neurological consequences at birth are irreversible and sometimes devastating resulting in paraplegia, hydrocephalus and hindbrain herniation among other sequelae[4,5].



Interestingly, some mothers appear to have a predisposition for pregnancies with NTDs. Mothers who have had an affected fetus have a 3% recurrence risk in any subsequent pregnancy, which rises to 10% after conceiving a second NTD embryo[6]. The predisposition to having children affected by NTDs has not been explained and may relate to differential surveillance by the maternal immune system. It is known that maternal lymphocytes traffic to the fetus throughout gestation without causing harm[7]. Their purpose is presumably to protect the fetus from environmental factors that may cause injury. It has been postulated that the maternal immune system is capable of providing surveillance for early fetal teratogenic events[8,9]. However, very little information exists regarding the types of maternal immune cells or cell signaling mechanisms that are needed for fetal protection. Understanding the differences in maternal immunologic phenotype at the maternal-fetal interface may be the key to discovering the etiology for the maternal predisposition to fetal NTD development. **To address this knowledge gap, we hypothesized that maternal cell trafficking to the fetal circulation is increased in response to abnormal development.**



Methods

Mating and NTD creation

We utilized a murine model of NTD development in utero. In order to create defects in the fetuses, pregnant dams were administered 600mg/kg VPA via IP injection at E8. Studies were performed in both immunologically matched (B6.Ly5.1 female x B6.Ly5.2 male) and immunologically mis-matched (B6.Ly5.1 female x Balb/c male) maternal-fetal hybrid matings.

Flow Cytometry

Peripheral blood from the mother was drawn prior to mating, prior to VPA administration and at harvest at day E14. Immediately after harvest, fetuses were quickly rinsed and cord blood was taken. Brain, liver, and musculoskeletal tissue was also harvested and flow cytometry performed of PMBCs evaluating for CD45.1 double positive maternal immune cells.

Results

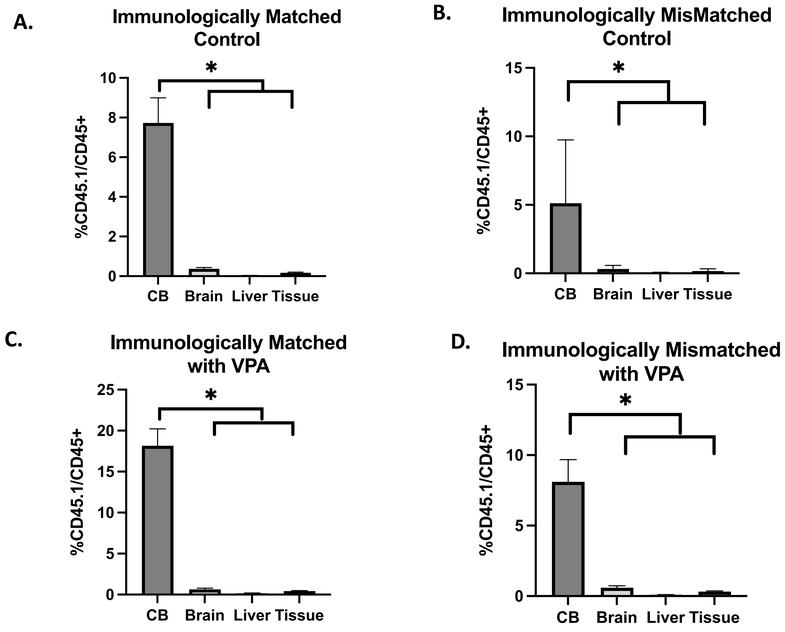


Fig. 1. Maternal cell trafficking in utero is tissue restricted both in normal and abnormal development. Graphical representation depicting higher rates of maternal cell trafficking in fetal cord blood compared to fetal brain, liver and musculoskeletal tissue in both (A) immunologically matched maternal-fetal pairings (7.7% vs 0.4%, 0.03%, 0.2%; p<0.05) and (B) immunologically mismatched maternal-fetal pairings that are untreated (5.2% vs 0.3%, 0.04%, 0.1%; p<0.05). Graphical representation depicting higher rates of maternal cell trafficking in fetal cord blood compared to fetal brain, liver and musculoskeletal tissue in both (C) immunologically matched maternal-fetal pairings (18.1% vs 0.6%, 0.2%, 0.4%; p<0.05) and (D) immunologically mismatched maternal-fetal pairings that are treated with VPA (8.1% vs 0.6%, 0.1%, 0.3%; p<0.05).

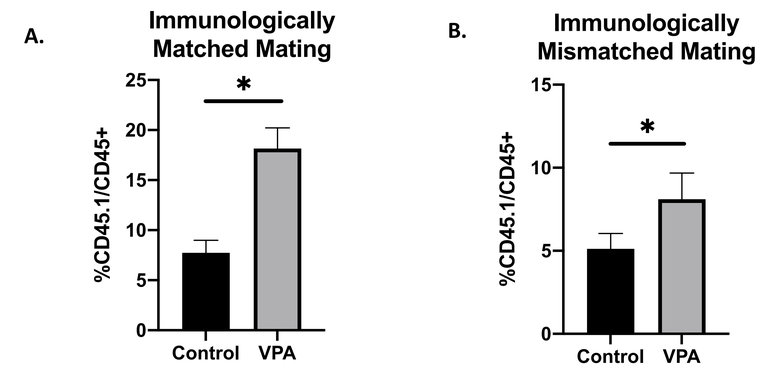


Fig. 2 More maternal cell trafficking in VPA treated litters compared to controls in both immunologically matched and mismatched pairings. (A) Graphical representation depicting higher rates of maternal cell trafficking in fetal cord blood in VPA treated litters compared to controls when the fetus and mother are immunologically matched (18.2% vs 7.7%; p<0.05). (B) Graphical representation depicting higher rates of maternal cell trafficking in fetal cord blood in VPA treated litters compared to controls when the fetus and mother are immunologically mis-matched (8.1% vs 5.1%; p<0.05).

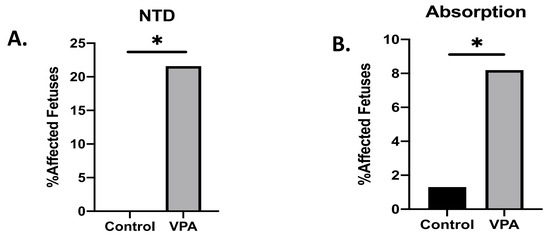


Fig. 3. NTD only seen in VPA treated litters. Significantly more fetal absorption in VPA treated litters compared to controls. (A) Graphical representation comparing the % fetuses affected by NTD in VPA treated dams vs controls (21.6% vs 0%; p<0.05). (B) Graphical representation comparing the % fetuses affected by absorption in VPA treated dams vs controls (1.3% vs 8.2%; p<0.05).

Conclusions

From these studies, we conclude:

- There are **much higher levels of maternal immune cells in fetal cord blood compared to fetal tissues** supporting that maternal cell chimerism is tissue-restricted in both normal and abnormal development.
- Importantly, the pattern was consistent between the immunologically matched and disparate maternal-fetal combinations suggesting that the **tissue-restriction was not related to immunologic disparity** but stems from differential trafficking or selective differences in the proliferation or survival of maternal cells.
- **Maternal cell trafficking to the fetus is significantly affected by abnormal fetal development.**
- **The higher rates of fetal resorption in abnormal litters support a link between maternal leukocyte trafficking and the surveillance of fetal fitness.**

Future experiments will clarify the specific cell phenotypes and mechanisms regulating this process.

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