Loss of Allospecific Activating Receptor Expressing NK Cells Results in Maternal Tolerance of the Aberrant Fetus

K.C. Ott 1, M. Oria 2, C.R. Redden 1, H.K. Kang 1, L.E. Turner 1, A.M. Alhajat 1, S. Duru 2, J.L. Peiro 2, A.F. Shaaban 1

1Center for Fetal and Cellular and Molecular Therapy, Ann & Robert H. Lurie Children’s Hospital of Chicago, Chicago, IL, 2Center for Fetal and Placental Research, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH

Abstract

Maternal survival provocatively "spontaneous" first trimester pregnancies without causing harm.1 It has been postulated that the maternal immune system is capable of providing surveillance for early fetal teratogenic events.2 However, very little information exists regarding the types of maternal immune cells or signaling mechanisms that are needed for fetal protection. Clinical studies have shown that mothers with high expression of NK cell allospecific activating receptors (NK-IAA’s) experience an unexplained high rate of first trimester spontaneous pregnancy loss.3 It remains unclear if these "spontaneous" losses result from direct interaction of maternal NK cells with an aberrant fetus leading to selective fetal absorption. In this report, we address the provocative hypothesis that loss of maternal NK-IAA’s will result in increased survival of fetuses with NTD’s.

Introduction / Methods

An inbred murine model of valproic acid (VPA)-induced fetal neural tube defects (NTD) performed of pregnant B6 (Group 1) and Balb/c (Group 2) dams was utilized. At E8, dams were given IP injection of 600mg/kg of VPA. In Group 3 maternal NK cells were selectively eliminated via IP injection of 200ug mAb PK136 on E7 in the B6 mice prior to VPA injection at E8. Finally, in Group 4 the NK activating receptor Ly49H was deleted using 200ug of the mAb 3D10 on E7 in the B6 mice prior to VPA injection at E8.

All litters were harvested on E14 and prevalence of NTD compared via dissecting microscope between each treatment group and saline treated controls. Maternal peripheral blood samples were taken prior to mating (E-2), prior to VPA injection (E8) and at harvest (E14). Flow cytometry was performed on these samples examining NK cell, T cell, Ly49H, Ly49D Ly49G, Ly49A, Ly49F and Ly49C levels.

Figure 1. B6 Litter after VPA Treatment. Here you can see a litter of 10 fetuses half of which are affected by NTD in the yellow box you can clearly see a neural fissure on the left with a closed diastem compared to a fetus with NTD on the right with an open diastem.

Figure 2. NK Depletion Results in Increased NTD Development. (A) Balb/c dams had a higher rate of NTD affected fetuses compared to B6 dams (p<0.05, p<0.05) (B) The prevalence of fetuses with NTD in B6 dams was increased after maternal NK depletion compared to dams treated with VPA alone (0.1% vs 14.5%, p<0.05)

Figure 3. NK B6 vs Balb/c

Figure 4. VPAH Depletion Results in Increased NTD Development. (A) Ly49H knockout was highly effective in reducing the frequency of NTD's in saline treated controls. NTD's in VPAH was twice as high in Ly49H-depleted dams when compared to controls given VPA alone (29.6% vs 14.5%, p<0.05). (B) Cisdeine was unaffected between the groups.

Conclusions

- Different strains display unique susceptibilities to NTD development in utero which follow the mother.
- Loss of maternal NK function overall results in higher rates of NTD development.
- Specifically, loss of Ly49H activating receptor predisposes to higher NTD rates in utero.
- These findings establish a functional link between expression of maternal AAR’s and maternal “tolerance” of the abnormal fetus.
- Further studies are needed to define mechanisms by which maternal NK cells recognize and prevent propagation of aberrant fetal development.

References