An E4BP4 knockout model to assess the distribution of uterine Natural Killer cell subsets in mouse pregnancy

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Abstract

Natural killer cells have been linked to a number of disorders of pregnancy by both mouse studies and association studies in humans, with NK cell dysfunction leading to reduced spiral artery remodelling. However, little research has centred on the importance of the newly divided subsets of uterine natural killer cells, some of which appear to develop independently of E4BP4, in spiral artery remodelling, and subsequently fetal growth. Hence, we sought to analyse the dependence of different uterine subsets of natural killer cells on E4BP4 by use of a knock out model. Our data show the E4bp4$^{-/-}$ mouse possesses uterine NK cells and may show an altered distribution of DBA^{+} NK cells compared to C57BL/6. Further investigation is needed to account for decreased vascular remodelling and fetal growth in this knockout model.

Methods

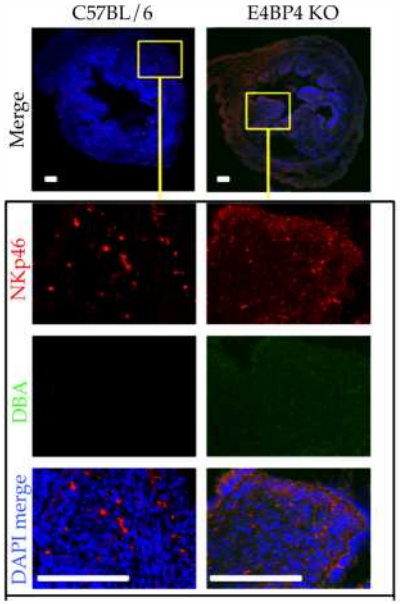
Mouse experiments were conducted under a Home Office License. C57BL/6 or E4bp4$^{-/-}$ virgin mice were introduced to males at 7 - 10 weeks. Timing of conception was determined by detection of a copulation plug representing g.d 0.5.

For colorimetric immunohistochemistry, NKp46 expression and DBA staining were visualized in 7 µm acetone fixed cryosections of uterine tissues. Images were scanned using a x40 objective on a Hamamatsu NanoZoomer scanner and analysed using the NDP View software.

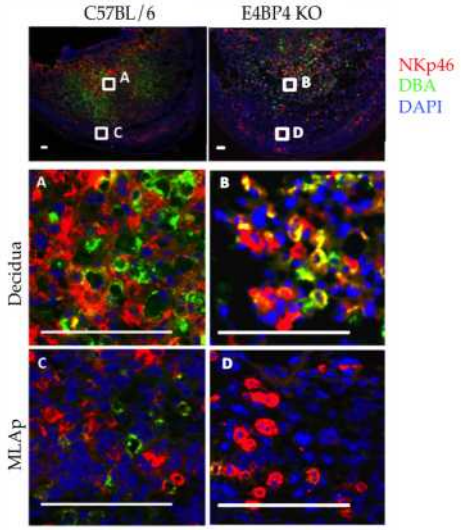
For immunofluorescent detection, NKp46 expression, DBA and DAPI staining were visualized in 7 µm thick acetone fixed cryosections of uterine tissues. Images were acquired on a Leica Ariol SL50 and analysed on Ariol software.

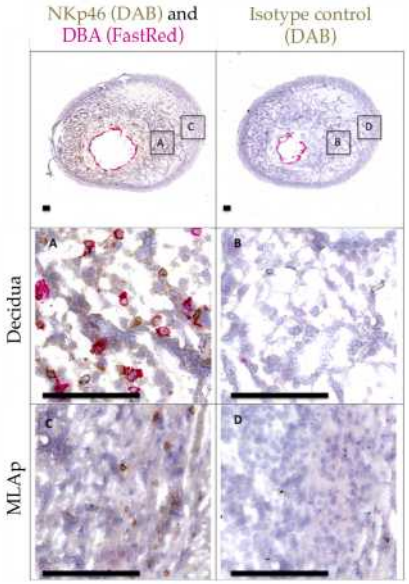
Results

Analysis of NK cell subsets in the virgin C56Bl/6 and E4bp4$^{-/-}$ uterus by colorimetric IHC (data not shown) and immunofluorescence (below) shows that DBA^{+}NKp46^{+} cells are absent but DBA^{-}NKp46^{+} present in both genotypes.



Immunofluorescent and colorimetric detection of DBA and NKp46 in the E4bp4$^{-/-}$ uterus at midgestation (g.d 9.5), show that DBA^{+}NKp46^{+} and DBA^{-}NKp46^{+} cells are present in the decidua basalis whereas only DBA^{-} cells may be visualised in the MLAp (mesometrial lymphoid aggregate of pregnancy) (below and right).





In contrast, in the B6 both DBA^{+} and DBA^{-} NKp46^{+} cells are seen in both the decidua basalis and MLAp. Moreover, Nkp46^{+} cells appear to be more abundant in the B6, although flow cytometry data or stereometry is needed to confirm this.

Discussion and Conclusions

E4BP4 has been found to be essential for the development of splenic NK cells leading to the hypothesis that E4BP4 dependence for development is a defining feature of NK cells.[1,2] Subsequently a number of tissues, including the uterus, have been shown to contain resident NK cells that are not dependent on E4BP4 for their development.[3] Here we confirm that the E4bp4$^{-/-}$ virgin uterus contains DBA^{-}NKp46^{+} cells, supporting the hypothesis that these cells develop along a pathway distinct to conventional NK cells.

Previously our lab found that the knockout shows decreased spiral artery remodelling and fetal weight compared to B6 mice.[4] The lack of E4BP4 may exert its effect either by absence of a subset or by altering the proportions of subsets in the pregnant uterus. Here we demonstrate that DBA^{+}NKp46^{+} cells are present at mid gestation in E4bp4$^{-/-}$ mice, as they are in the B6, but appear to be less abundant. Hence further work is needed to assess whether other subsets are absent and to quantitate the proportions of subsets.

Whilst this work is in its early stages, given the evidence implicating NK cells in common disorders of pregnancy (pre-eclampsia, IUGR and recurrent miscarriage) characterisation of the pathways regulating the cells responsible for these phenotypes may be of future clinical significance.[5]

References

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