



IL-27 AND IMMUNOSUPPRESSION IN INFANTS

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Abstract

Background and Significance: Successful human pregnancy depends on the initiation and maintenance of local immunological tolerance to the semi-allogenic fetus. The immunosuppressive microenvironment at the fetal-maternal interface also regulates the fetal immune system and thus may at least be partially responsible for the immaturity of the neonatal immune system. The resultant deficiencies lead to a greater susceptibility to microbial infection, a major cause of mortality early in life. Studies in our laboratory have shown that human cord blood-derived macrophages express a higher level of interleukin (IL)-27 compared to adult macrophages. IL-27 has been shown to oppose inflammation and may be induced during pregnancy to regulate the maternal immune response to the fetus. To determine whether this elevated expression is maintained throughout infancy, we examined the expression of IL-27 throughout the murine life cycle along with possible mechanisms of immunosuppression.

Methods: Spleens were harvested from C57BL/6 mice ($n = 6$) at different stages of life and homogenized for RNA isolation. Gene expression of both subunits of IL-27, EBI3 and p28, was measured by real time PCR. To confirm gene expression levels, spleen-derived macrophages from mice ($n = 4$) were immunolabeled for IL-27 protein at three stages of life. Macrophage surface marker and intracellular IL-27 were concurrently labeled with fluorophore-conjugated specific antibodies for flow cytometry analysis.

Results: The relative gene expression of IL-27 in adult (day 56) mice was significantly lower than the expression in neonatal (day 8) mice. Similar results were found in human cord-blood derived macrophages, which express IL-27 at an elevated rate compared to adult macrophages. Age-dependent analysis of murine IL-27 gene expression throughout the mouse life cycle indicated that levels of IL-27 remain significantly elevated through infancy and adolescence. Flow cytometry analysis further confirmed these results, demonstrating that macrophage IL-27 protein levels are significantly higher in both neonate and infant mice compared to adult mice.

Discussion: The elevated expression of IL-27 throughout infancy in the mouse model indicates that increased levels of IL-27 may contribute to the immunosuppressive phenotype of neonatal macrophages. Thus susceptibility to infection that is observed throughout early life may be minimized by blocking IL-27. Progesterone directly induces transcription of IL-27 in a dose-responsive manner, which may be beneficial during pregnancy. However, the mechanism of continued elevated expression of IL-27 in infancy must be examined further.

Background

- IL-27 is a heterodimeric cytokine that consists of the Epstein-Barr virus-induced gene 3 (EBI3) and IL-27p28 proteins (1).
- The anti-inflammatory activity of IL-27 includes the generation of anti-inflammatory T cells (Tr1) that produce large amounts of the immunosuppressive cytokine IL-10 (2), and the suppression of T_H17 cells that mediate inflammation (3).
- Studies in our laboratory have shown that human cord blood-derived macrophages (Mφs) express a higher level of interleukin (IL)-27 compared to adult Mφs (Figure 1).
- Our hypothesis is that IL-27 expression is elevated at the fetal-maternal interface to ensure maternal immunological tolerance of the fetus, but that this immunosuppressive state extends into the early neonatal period and contributes to neonatal immune deficiency and increased susceptibility to infection.

Figure 1. IL-27 Expression in Cord Blood and Adult Mφs

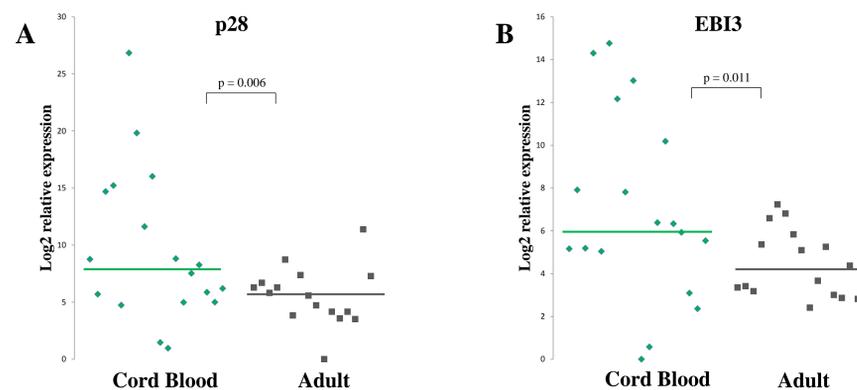


Figure 1. RNA was isolated from cord blood Mφs ($n=18$) or Mφs from adult donors ($n=16$). Gene expression of both subunits of IL-27, (A) p28 and (B) EBI3, was measured by real time PCR and represented as \log_2 expression. Each symbol represents an individual donor and the mean of each data set is denoted with a line. IL-27 expression was compared using a nonparametric Mann-Whitney test. The results are significant at the indicated p values in the 95% confidence interval.

Objective

The purpose of this study was to:

- determine if IL-27 expression remains elevated throughout infancy

Methods

A mouse model was used to determine levels of IL-27 expression throughout life:

- Spleens were harvested from C57BL/6 mice ($n = 6$) at different stages of life and homogenized for RNA isolation.
- Gene expression of both subunits of IL-27, EBI3 and p28, was measured by real time PCR.

To confirm gene expression levels:

- Spleen-derived macrophages from mice ($n = 4$) were immunolabeled for IL-27 protein at three stages of life.
- Macrophage surface marker and intracellular IL-27 were concurrently labeled with fluorophore-conjugated specific antibodies for flow cytometry analysis.

The IRB approval designation for this study is PH-2011-066; Pro00012437.

Results

Figure 2. Age-Dependent Murine IL-27 Gene Expression

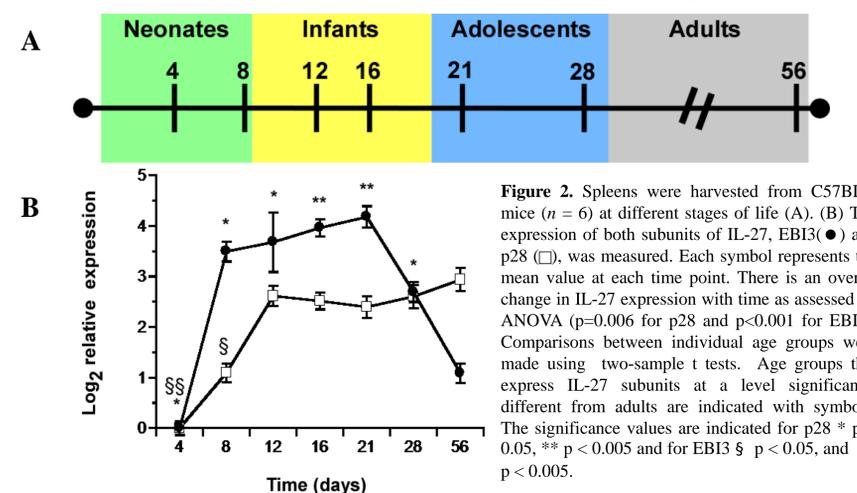


Figure 2. Spleens were harvested from C57BL/6 mice ($n = 6$) at different stages of life (A). (B) The expression of both subunits of IL-27, EBI3 (●) and p28 (□), was measured. Each symbol represents the mean value at each time point. There is an overall change in IL-27 expression with time as assessed by ANOVA ($p=0.006$ for p28 and $p<0.001$ for EBI3). Comparisons between individual age groups were made using two-sample t tests. Age groups that express IL-27 subunits at a level significantly different from adults are indicated with symbols. The significance values are indicated for p28 * $p < 0.05$, ** $p < 0.005$ and for EBI3 § $p < 0.05$, and §§ $p < 0.005$.

Results Continued

Figure 3. IL-27 Age-Dependent Protein Comparison in Mice

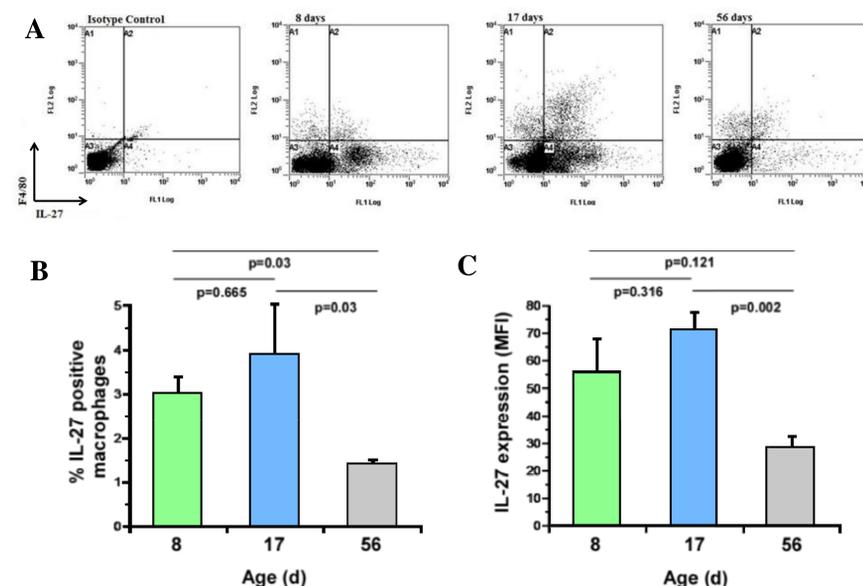


Figure 3. (A) Murine splenocytes ($n = 4$) from neonatal, infant, and adult mice were immunolabeled for IL-27 protein and a Mφ surface marker (F4/80). (B) The percentage of labeled Mφs that expressed IL-27 (non-normally distributed data) was analyzed with the Mann-Whitney test, and (C) the level of IL-27 expressed by the Mφ population (mean fluorescent intensity; MFI) was analyzed with the two sample t-test.

Conclusions

- IL-27 expression remains elevated throughout infancy and into adolescence in the mouse model.
- IL-27 protein levels are significantly higher in both neonates and infants compared to adults in the mouse model.
- Increased levels of IL-27 may contribute to the immunosuppressive phenotype observed in neonatal macrophages and thus the increased susceptibility to infection that is observed throughout early life.

Acknowledgements

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References

- Devergne, O., Hummel, M., Koeppen, H., LeBeau, M. M., Nathanson, E. C., Kieff, E., and M. Birkenbach. (1996) A novel interleukin-12 p40 related protein induced by latent Epstein-Barr virus infection in B lymphocytes. *J. Virol.* 70:1143.
- Awasthi A., Carrier Y., Peron J. P. S., Bettelli, E., Kamanka, M., Flavell, R. A., Kuckroo, V. K., Oukka, M., and H. L. Weiner. (2007) A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory cells. *Nature Immunol.* 8:1380-9.
- Stumhofer J. S., Laurence A., Wilson E. H., et al. (2006) Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system. *Nature Immunol.* 7:937-45.