Immune Ontogeny and Engraftment Receptivity in the Sheep Fetus

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Abstract

The therapeutic application of in utero hematopoietic stem cell (HSC) transplantation (IUHSCT) is theoretically attractive for definitive treatment of congenital disease states. Investigating this technique in sheep, we have previously shown long-term engraftment and expression of both allogeneic and xenogeneic donor cells without cytoablation and, under appropriate conditions, without GVHD. The theoretical basis for IUHSCT is the well-recognized immune receptivity of the fetus to engraftment of donor cells. Engraftment and long-term expression of donor human and allogeneic sheep HSC reliably occur in the fetal sheep model if the IUHSCT is performed prior to day 71 of gestation (term: 145 days), during the period of presumed immuno-naïveté. Investigations using alternate animals have also noted that gestational age at transplantation is critical to achieving long-term engraftment, presumably as a result of inducing durable immune tolerance to the donor. Despite this presumption, however, the biologic explanation for fetal receptivity to donor engraftment and subsequent long-term tolerance following transplantation early in gestation is not known. In the present studies, we investigated the role fetal immune ontogeny plays in the induction of tolerance following IUHSCT in sheep. To this end, we performed parallel experiments examining engraftment receptivity of fetal sheep to allogeneic and xenogeneic HSC and the appearance of immune phenotypes in fetal sheep lymphoid organs at varying gestational ages (days 39 to birth), attempting to draw correlations between the appearance/absence of specific immune cells and the ability to achieve durable engraftment and immune tolerance. Engraftment receptivity was determined 60 days post-transplantation at different time points in sheep fetal gestation, while immune phenotypes were determined by flow cytometry using commercially available antibodies to immune cell surface markers. Our results indicate that the fetus is largely non-receptive to engraftment of both allogeneic and xenogeneic donor HSC prior to day 52 gestation and possesses a peak in engraftment receptivity between days 64-71 of gestation, which rapidly declines thereafter. With respect to the developing fetal immune system, the period of peak engraftment receptivity was associated with the expression of CD45 on all cells in the thymus. Double-positive and single-positive CD4 and CD8 cells began appearing in the thymus just prior (day 45 of gestation) to the beginning of the engraftment window, while single-positive CD4 or CD8 cells did not begin appearing in peripheral organs until late in the engraftment period, suggesting deletional mechanisms predominate during this time. In a similar fashion, surface IgM (sIgM)+ cells in the thymus were the first to express CD45, commencing expression around day 45 of gestation, with a comparable delay in the appearance of IgM+/CD45+ cells in the peripheral blood and spleen until late in the engraftment window. These findings support a central role for the thymus in multilineage immune cell maturation during the period of fetal transplantation receptivity. Further, they suggest that fetal engraftment receptivity/long-term engraftment and expression following IUHSCT is due to gestational age-dependent deletional tolerance. Further, our findings suggest that IUHSCT in humans may be more successful if performed during the comparable period in human gestation.

Experimental plan

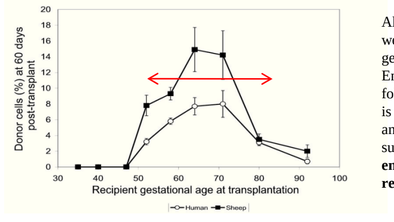
Fetal sheep engraftment and immune ontogeny was studied in parallel.

Engraftment:
In utero transplantation was performed using allogeneic and xenogeneic donor cells. Engraftment was determined 60 days following transplantation in the bone marrow.
Allogeneic donor cells: $4X10^6$ T depleted sheep cord blood of different sex or different hemoglobin.

Xenogeneic donor cells: $2X10^6$ CD34 purified normal human bone marrow.
Fetal transplantation was performed at the following gestational ages: 35, 40, 47, 52, 58, 64, 71, 80 & 92 days as previously described

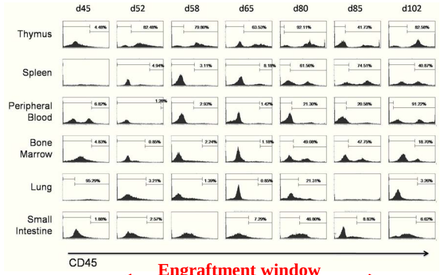
Immune ontogeny:
Flow cytometry was performed on single cell suspensions of thymus, spleen, peripheral blood, bone marrow, lung and small intestine using antibodies to sheep surface CD4, CD8, IgM & CD45 at the varying gestational ages identified.

Engraftment

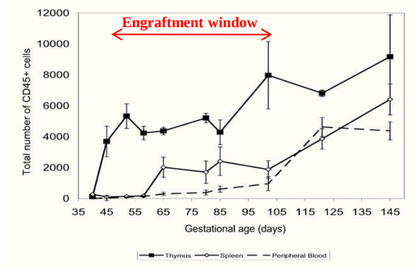


Allogeneic or xenogeneic donor cells were transplanted at the varying gestational ages identified. Engraftment was determined 60 days following transplantation. Engraftment is not noted prior to day 52 gestation and dissipates at day 92 gestation. This supports the presence of an **engraftment window (double headed red arrow)** in the developing fetus.

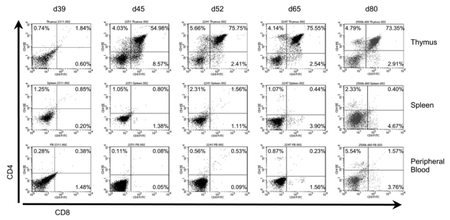
Immune ontogeny: CD45 expression

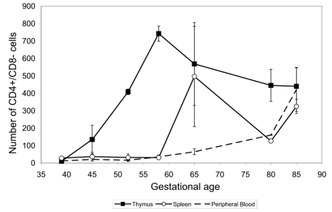


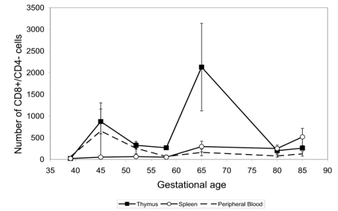
Engraftment window



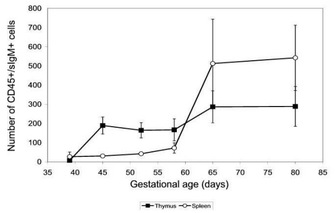
Immune ontogeny: CD4 & 8 expression







Immune ontogeny: IgM & CD45 expression



Conclusions

1. **Pleural disease is common in patients with rheumatoid arthritis although usually of little or transient clinical significance. Severe longstanding disabling pleural disease is uncommon although empyema is a common complication.**
2. **Our patient had longstanding pleural disease not responsive to methotrexate and tumor necrosis factor inhibition.**
3. **Pathologic findings included a thickened fibrotic pleura with evidence for chronic inflammation, palisaded histiocytes and multinucleated giant cells all characteristic of rheumatoid pleural disease.**
4. **Rituximab treatment resulted in symptomatic improvement, closing of the open wound that remained long after chest tube removal. Laboratory improvement was also noted.**
5. **B cells (CD19 & CD20+ cells) were not seen at 6 and 10 months following rituximab therapy. Circulating immunoglobulin levels were minimally affected following rituximab therapy (data not shown).**
6. **The role of rituximab in treating extra-articular manifestations of rheumatoid arthritis should be explored.**

References and Acknowledgements

1. Lake FR. Overview of lung disease associated with rheumatoid arthritis. Ed. Flaherty KR (Section editor) & Hollingsworth H (Deputy editor) UpToDate Online 6.1.
2. Jones FL, Blodgett RC. Empyema in rheumatoid pleuropulmonary disease. Annals of Internal Medicine (1971) 74: 665-71.
3. Yarbrough JW, Scaly WC, Miller JA. Thoracic surgery problems associated with rheumatoid arthritis. The Journal of Thoracic and Cardiovascular Surgery (1975) 69: 347-54.
4. Aru A, Engel U, Francis D. Characteristic and specific histological findings in rheumatoid pleurisy. Acta Pathologica, Microbiologica et Immunologica Scandinavia, Section A, Pathology. (1986) 94: 57-62.
5. Pixley JS, Wolf BJ, O'Neill W. Membranous glomerulopathy associated with rheumatoid arthritis may respond to rituximab. Scandinavian Journal of Rheumatology (in press).
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