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- Identification of Genes Responsible for Immunologic Tolerance
- Immunologic Tolerance in a Large Animal Model
- Lymphocyte Development and Differentiation: The Role of the Notch Gene

AWARDS

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Transplantation has matured to become the treatment of choice for end-stage renal and hepatic disease. Despite many advances in immunosuppression, the majority of transplant grafts continue to be lost to immunologic causes. Of these, rejection, a lymphocyte-mediated response to foreign tissue, is a leading factor. Our research is directed toward understanding the factors responsible for this immune response and developing techniques to abrogate them. Our laboratory focuses on the control

of development and differentiation as it pertains to lymphocytes and hematopoietic stem cells. We are using large-scale cDNA array techniques to ascertain the regulatory genes involved in these processes. In addition, we are interested in developing tolerance strategies in a large animal transplant model using knowledge gained from this work.

Identification of Genes Responsible for Immunologic Tolerance

Tolerance describes a state of 'donor-specific unresponsiveness.' This develops *de novo* in some patients after organ or stem cell transplant. The events responsible for this have not been elucidated and would provide important insights into therapies that would mitigate the effects of chronic non-specific immunosuppression. We are actively seeking answers to these questions by employing cDNA arrays (Fig. 1) of lymphocyte subsets both from human patients who have developed tolerance and in a transgenic mouse model of tolerance. We expect that the patterns of gene expression novel to the tolerant versus the non-tolerant state could provide a tool to determine when the tolerant state is reached. Additionally, individual genes that are differentially regulated between these two states may lead to insights into the mechanisms of tolerance induction.

Immunologic Tolerance in a Large Animal Model

This part of our work involves a large animal transplant model. In collaboration with Drs. Rainer Storb and Beverly Torok-Storb at the Fred Hutchinson Cancer Research Center, we have successfully created dog models that are hematopoietic chimeras through hematopoietic stem cell transplantation. These animals have accepted renal transplants in the absence of immunosuppression from their DLA matched littermate donor, and currently have excellent renal function more than one year after renal transplantation (Fig. 2). We

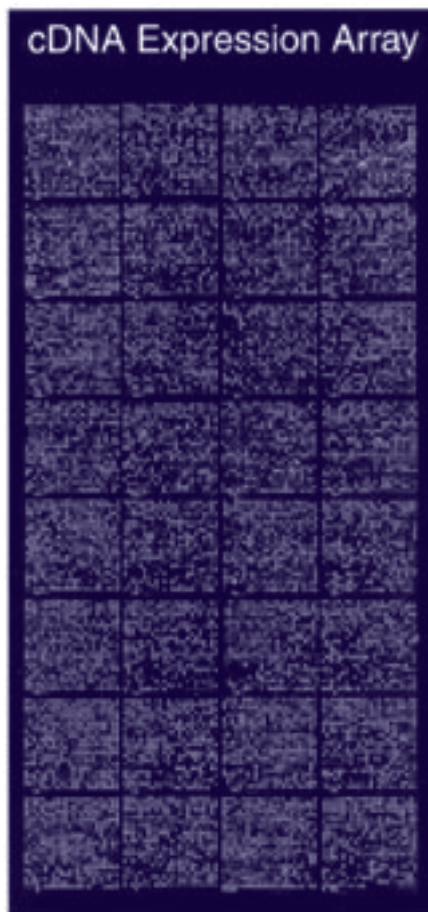


FIGURE 1

Understanding the control of lymphocyte development and maturation will lead to important insights into antigen specificity and immune dysregulation, and could be exploited to alter the immune response.

are exploiting this animal model to examine both the induction of tolerance and the robustness of hematopoietic chimerism as platform for organ transplantation in the absence of immunosuppression.

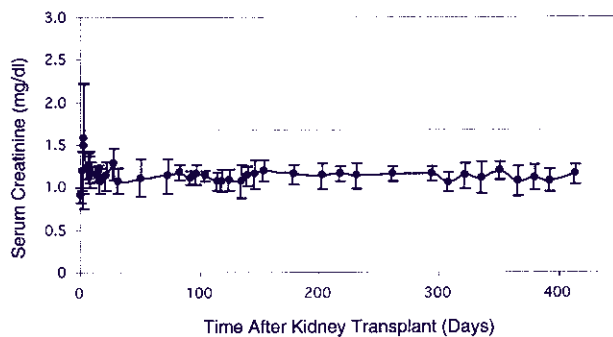


FIGURE 2

Lymphocyte Development and Differentiation: The Role of the Notch Genes

Lymphocyte development proceeds along a pathway characterized by a series of gene rearrangements that impart antigen specificity. Alterations in these pathways can contribute significantly to the development of autoimmune and immunodeficiency states. Understanding the control of lymphocyte development and maturation will lead to important insights into antigen specificity and immune dysregulation, and could be exploited to alter the immune response.

One phylogenetically conserved family of transmembrane receptors with known importance in cell-fate decisions is Notch. Originally identified in *Drosophila melanogaster*, Notch family members subsequently have been identified in other invertebrates, and four mammalian homologues are now known. Their function involves control of developmental cell-fate decisions through Notch receptor signaling which is thought to delay or block differentiation of uncommitted cells. The mammalian Notch family members are ubiquitously expressed and all are expressed in lymphoid tissue. Notch1 has been shown to influence the development of T lymphocytes, and Notch2 has recently been found to inhibit a transcription factor (E47) that is necessary for B lymphocyte. Our preliminary work shows that while the Notch family members are expressed in developing B lymphocytes, Notch2 expression is highest, suggesting unique activity in this cell population.

The focus of our work is to determine the role that Notch family members have in controlling lymphocyte development. To this end we have generated mice which overexpress the constitutively active intracellular portion of the Notch2 and Notch3 genes. We are currently analyzing the animals to characterize the phenotypic changes resulting from increased Notch activity. We plan to make use of cDNA array technology to identify downstream effectors of Notch, which remain to be fully characterized in mammals.

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