# Differential Gene Expression in the Thymus to Establish Low Toxicity Chemotherapeutic Agents

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# Immunotoxicity of Chemotherapeutic Agents

- Many chemotherapeutic agents decrease immune function
- Thymic damage may also occur, especially when used as a preparative agent for transplantation
- Thymic recover is often the rate limiting process in full immunological recovery
- Individual effects of chemotherapeutic agents on the thymus are largely unknown

# Thymic Function

- Rapid restoration of thymic derived T cells is vital to the prevention of immune-impaired conditions including cancer relapse, infection, and graft-versus-host disease (GVHD) following hematopoietic stem cell (HSC) transplantation

- Rapid thymic renewal correlates with thymic epithelial cell (TEC) recovery:

  - Identification of chemotherapeutic agents that damage TEC may lead to strategies to enhance thymic recovery, T cell production, and T cell diversity following chemotherapy treatment

## Hypothesis:

Gene Expression, as measured by mRNA production, will reflect the functional status of the thymus

# Thymic Function Monitored by Gene Analysis

If we can establish a panel of genes that are affected by or responsible for thymic function:

1. Can monitor thymic health
2. Establish agents that spare thymic damage
   e.g. Chemotherapeutic agents
3. Establish agents that can increase thymic function

# Gene Selection Criteria:

1. Genes expressed predominately in the thymus

1. Emphasis on genes expressed in TEC

2. Genes known to be important in T cell generation and thymic renewal
   e.g. CCL25, FoxN1

# Panel of Genes Expressed in Thymus

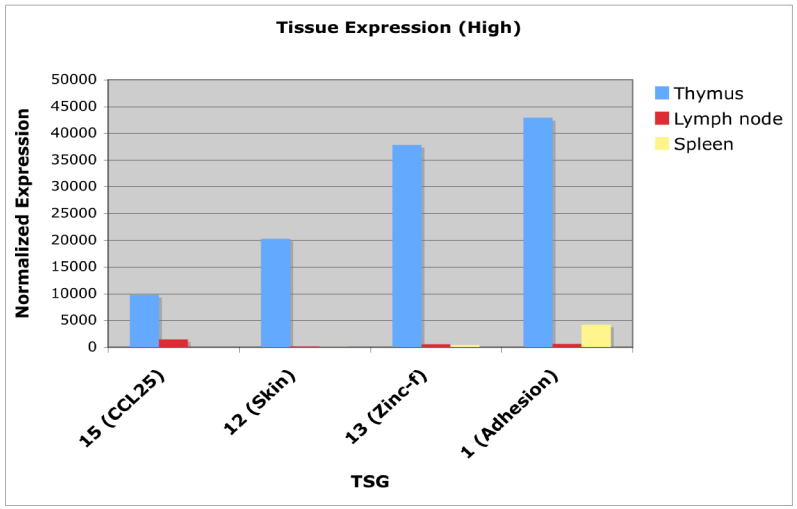
| ID | Gene |
|---|---|
| 1 | Adhesion/Matrix |
| 2 | Proteasome |
| 3 | TNFR-like |
| 4 | Plasmodium Related |
| 5 | Neur-AcetyR |
| 7 | Ubiquitin-like |
| 8 | Granule Marker |
| 9 | F-Box-like |
| 10 | CCL20 |
| 11 | Thy/Testis Expression |
| 12 | Thy/Skin Expression |
| 13 | Zinc-Finger-like |
| 14 | Thy/Liver specific |
| 15 | CCL25 |

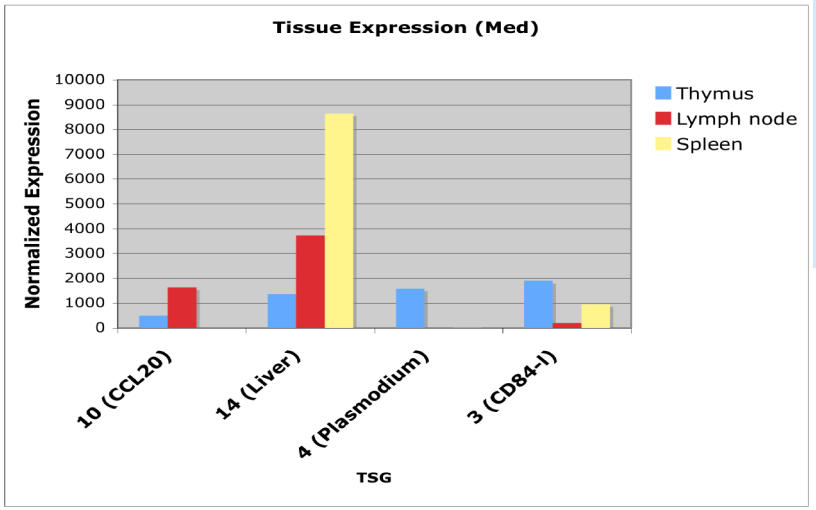
| ID | Gene |
|---|---|
| 16 | p38 |
| 17 | CDK2 |
| 18 | IFNg |
| 19 | CDX2 |
| 20 | GAPDH |
| 21 | CXCL12 |
| 22 | TBATAv6 |
| 23 | TBATAv5 |
| 24 | FoxN1 |
| 25 | LCK |
| 26 | LY51 |
| 27 | EpCam |
| 28 | AIRE |

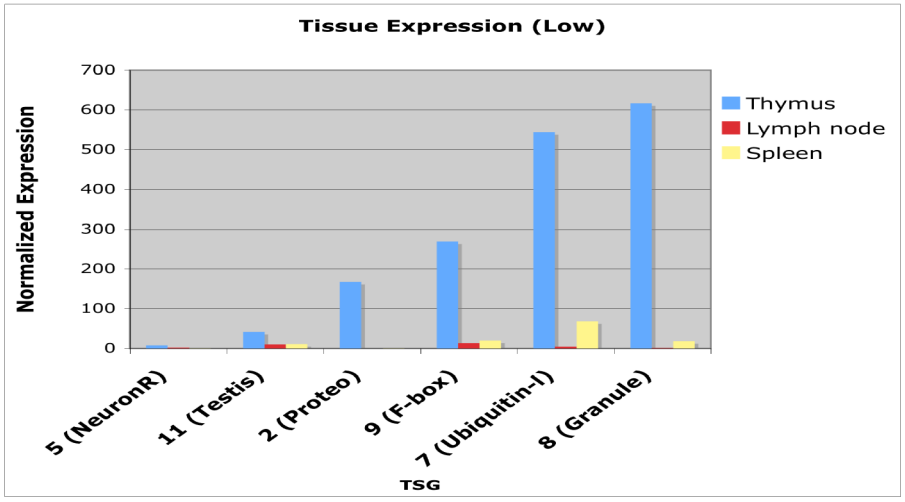
# Methods

- Irradiated (750 R) or cyclophosphamide treated (100mg/Kg) and age-matched control C57BL/6 female mice were evaluated
- Thymocytes were analyzed by flow cytometry analysis
- TEC were isolated using a mild protease digestion.
- Gene Expression analyzed by qRT-PCR

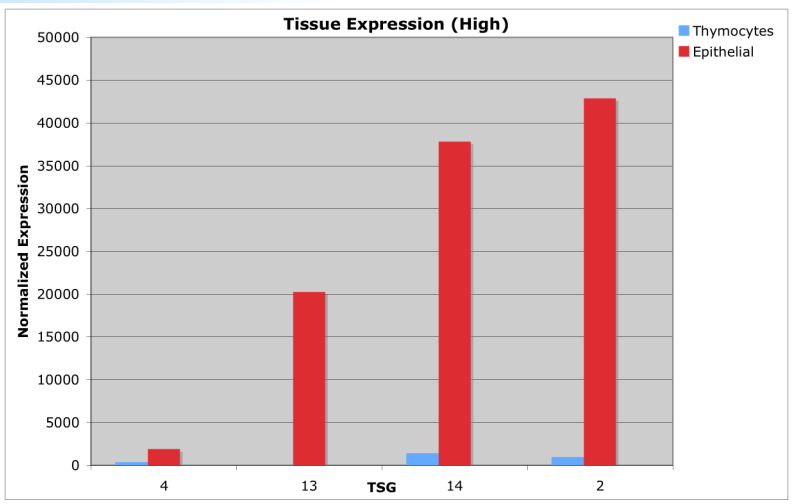
# Tissue Specificity of Thymic Genes

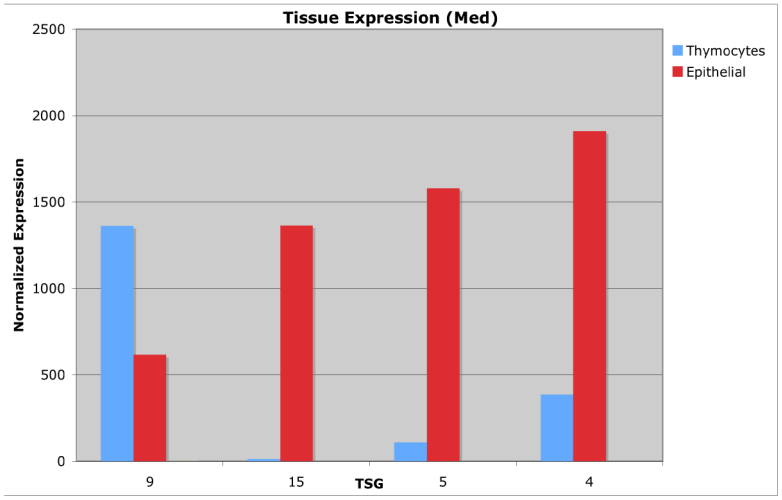


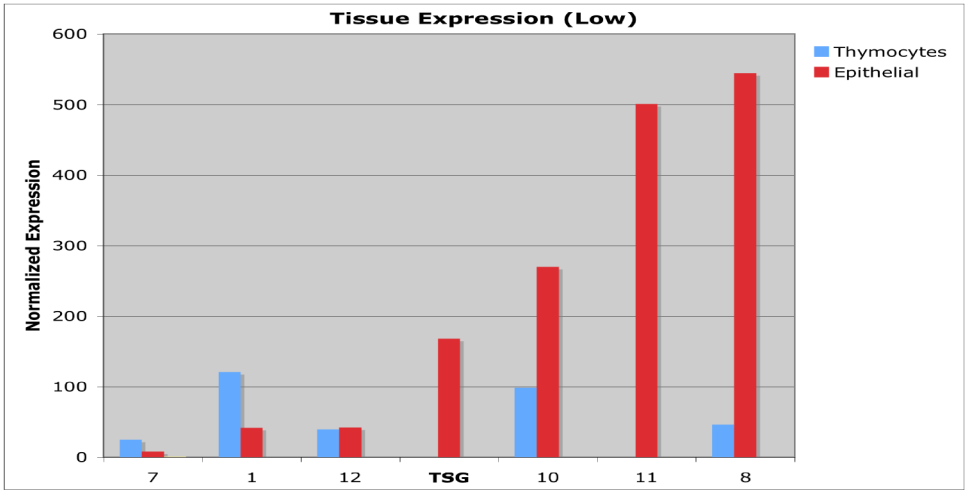



# TEC versus Thymocyte Expression

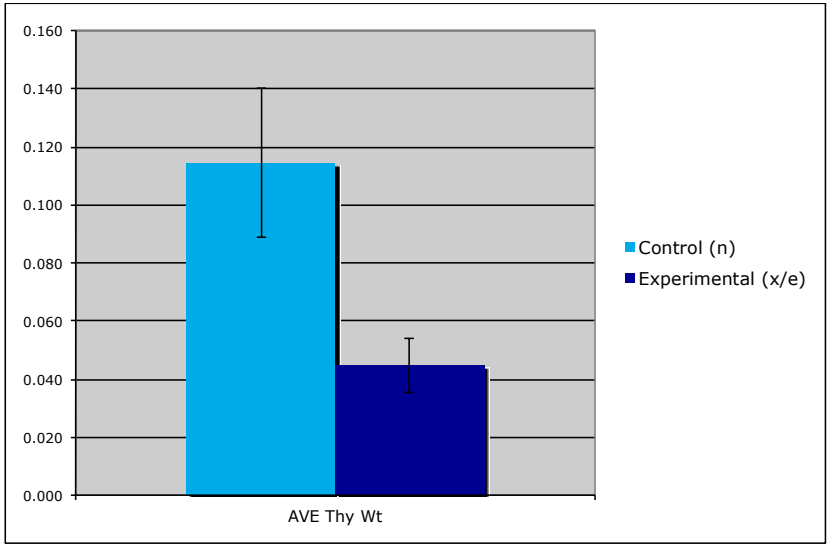


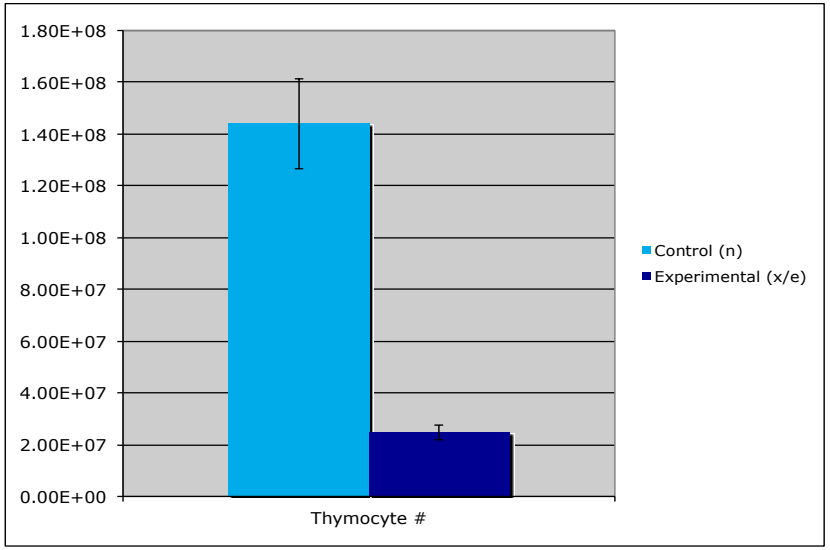



# Effect of Irradiation on Thymus

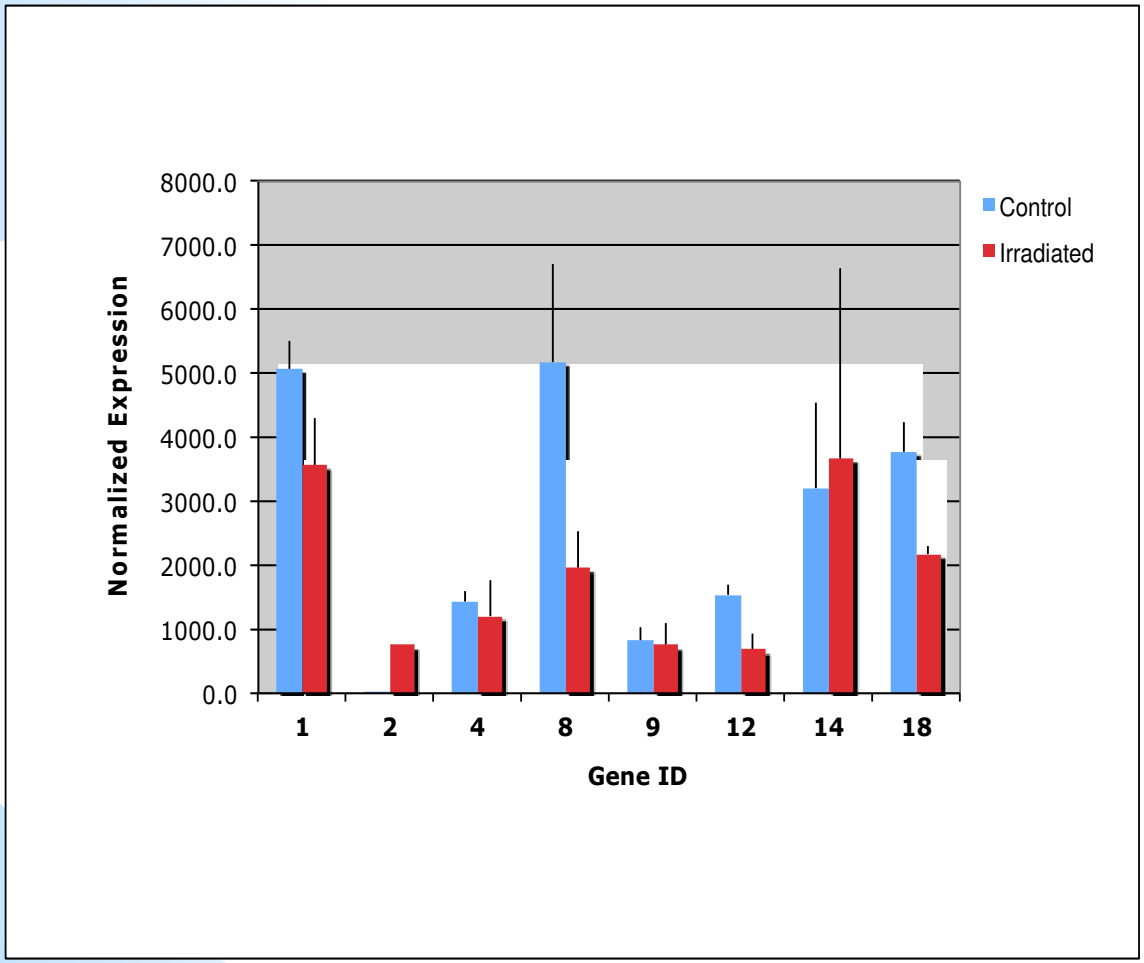
## Thymic Weight

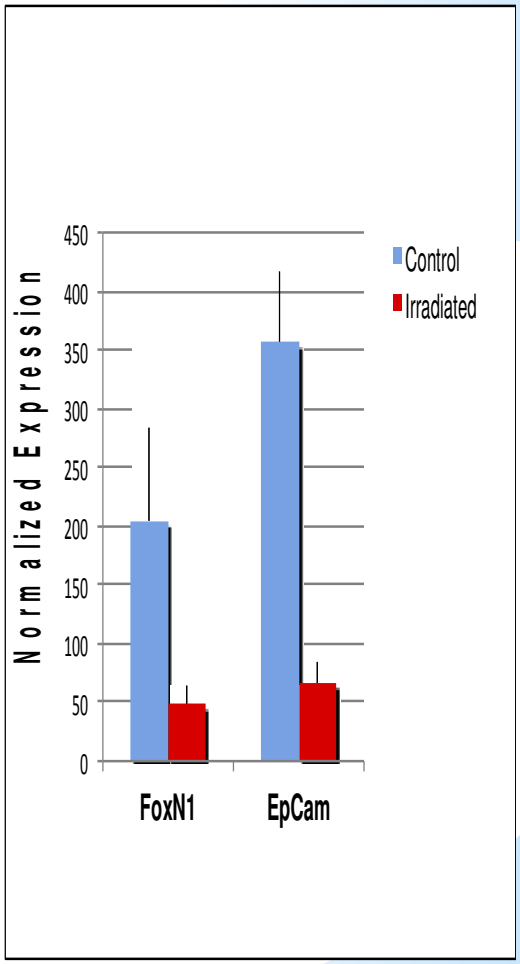



## Thymocyte Total Number

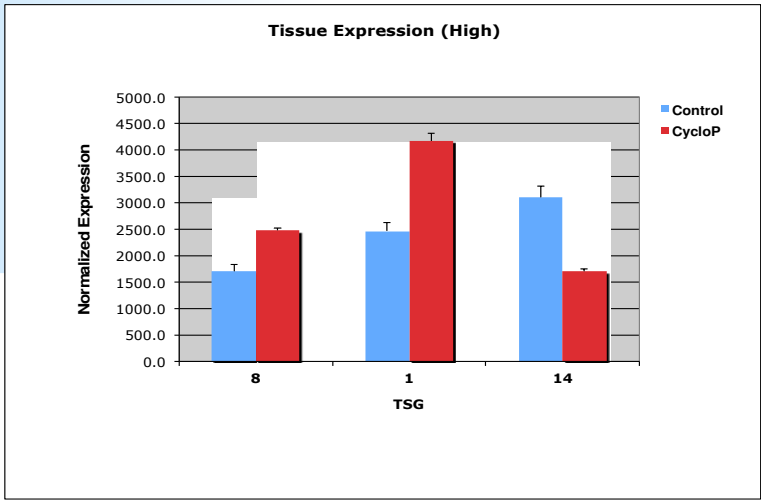


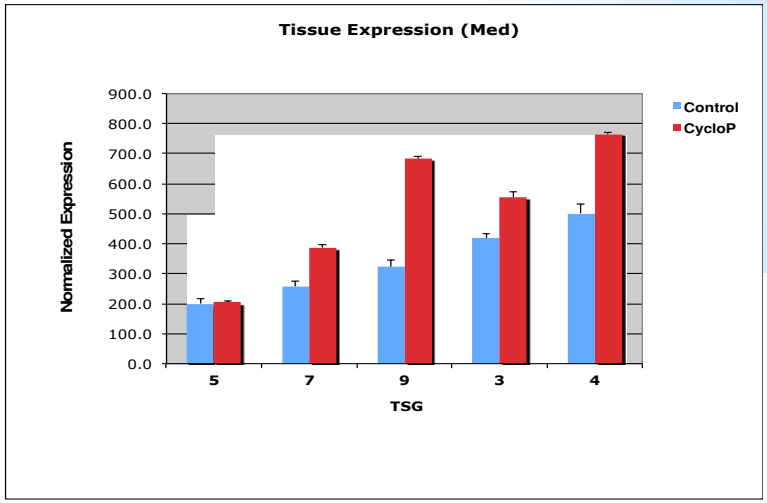
# Effect of Irradiation on Thymic Gene Expression

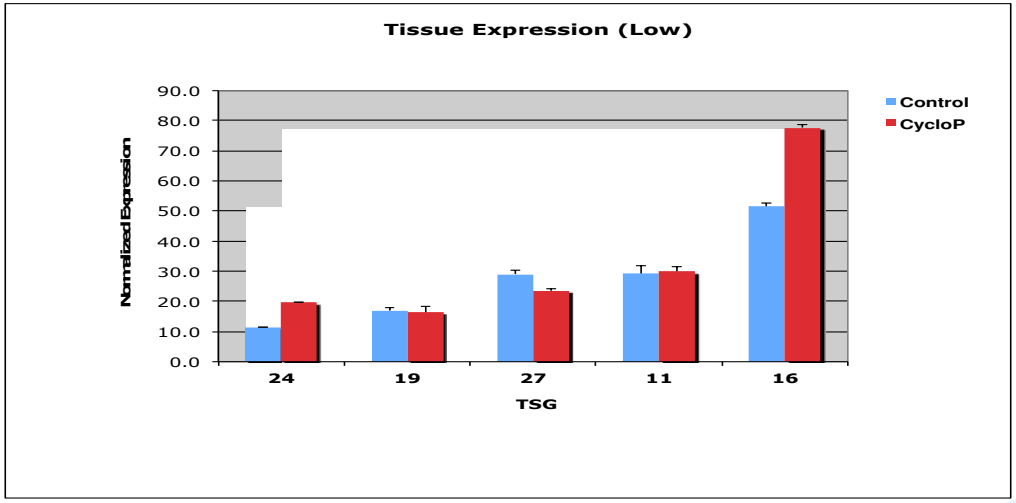


# Effect of Cyclophosphamide on Gene Expression



# Summary

Thymic recovery can be a rate limiting process in full immunological recovery following chemotherapy, thus establishing an in vivo model to detect thymic damage would be a useful tool to establish new therapeutic regimens.

Thymic epithelial cells (TEC), which have been shown to be essential for thymic recover, were the main target for our gene expression evaluation.

Using irradiation, a known thymic damaging agent, we demonstrate that our qRT-PCR specialized gene array was able to detect significant changes in the TEC population.

Similarly, we show that many of the genes in the gene panel were statistically altered following cyclophosphamide treatment, supporting our hypothesis that we can use this gene array to predict possible TEC changes.

# Contributors

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