



## TPRM Summer Student Final Presentations

**Thursday, August 26<sup>th</sup>, 2010**

**Music Room; Hart House – 7 Hart House Circle, University of Toronto**

### **7:45 – 8:15 Breakfast**

8:15 – 8:25 Opening Remarks – Dr. Gary Levy (Program Director, TPRM)

8:25 – 8:30 Presentation Reminders – Cheryl Bodnar (Master Educator, TPRM)

### **Student Presentations**

8:30 – 9:00 Daniel Pang - FcγRIIIa and FcγRIIIb gene polymorphisms and its implications in hepatitis C virus (HCV) infection.

9:00 – 9:30 Mitchell Harding – Optimization of FGL2 Monoclonal Antibody Production from Hybridoma Cells

9:30 – 10:00 Michael Law – Optimization of sFGL2 Production from Concanavalin A Stimulated Mice Splenocytes

### **10:00 – 10:15 Coffee Break**

10:15 – 10:45 Matt Timmons - Do Palliative Care Instruments Predict Heart Failure Severity When Compared to Standardized Heart Failure Instruments?

10:45 – 11:15 Calvin Lung - Bone Marrow-Derived Lung Progenitor Populations as Biomarkers for Human End-Stage Lung Disease Severity

11:15 – 11:45 Matthew Iu - An Orthotopic Mouse Model of Hepatocellular Carcinoma

11:45 – 12:15 Alicia Ali - The effect of immunosuppressant drug therapies targeted against human hepatocellular carcinoma cell lines

### **12:15 – 1:00 Lunch**

1:00 – 1:30 Alborz Borjian - The Role of Chemokines and Chemokine Receptors in Avian B-cell Development

1:30 – 2:00 Natalie Yavorska - Characterization of the Role of FGL2 in the Generation of a B-cell Response to Lymphocytic Choriomeningitis Virus (LCMV) WE

2:00 – 2:30 Anastasia Pargaru - Natural Killer Cell Characterization in a Tolerized Allograft Model

2:30 – 3:00 Caroline Jeon - Interferon gamma signaling in *lpr* double negative T cells is required for their ability to kill syngeneic alloreactive CD4<sup>+</sup> T cells.

**3:15 – 4:00 Softball Game: Staff vs. Students**

## **Fc $\gamma$ R1a and Fc $\gamma$ R1b gene polymorphisms and its implications in hepatitis C virus (HCV) infection.**

*Daniel Pang, Ahmed Helmy, Cheryl Bodnar, Nazia Selzner, Gary Levy*

*Multi-organ Transplant Program, Toronto General Research Institute, University Health Network, University of Toronto, Toronto, ON, Canada.*

Fc gamma receptors (Fc $\gamma$ R) on human myeloid and lymphoid cells play a pivotal role in immunoregulation through binding with immunoglobulin G (IgG). Their interaction generates potent cellular effector functions and serves as a link between the innate and adaptive immunity. Single nucleotide polymorphisms (SNPs) have been detected in the genes encoding for Fc $\gamma$ R1a and Fc $\gamma$ R1b, generating significant implications in disease pathogenesis. Fc $\gamma$ R polymorphism has not been previously studied in HCV infection, which affects approximately 200 million worldwide and is one of leading causes of hepatocellular carcinoma and liver transplantation in North America. We investigated the role of two allelic dimorphisms, histidine/arginine-131 of Fc $\gamma$ R1a and isoleucine/threonine-232 of Fc $\gamma$ R1b, in HCV susceptibility, disease severity and treatment response. 63 chronic HCV patients were enrolled in the study and were compared to established healthy control groups in literature (Chen et al, 2006; Duitus et al, 1995). Genomic DNA was extracted from peripheral blood and conventional PCR was performed to amplify the segment of interest, followed by DNA sequencing or gel electrophoresis to detect allelic types. Preliminary results for FCgR2A show 29/61(47.5%) patients are heterozygous (His/Arg), 14/61(23%) are homozygous (His/His) and 18/61(29.5%) are homozygous (Arg/Arg). For FCgR2B, 57/61(93.5%) patients are homozygous (Ile/Ile), 3/16 (4.9%) are heterozygous (Ile/Thr) and 1/61(1.6%) is homozygous (Thr/Thr). Preliminary findings show that SNPs are a common finding in HCV patients that may predict susceptibility and may serve as a basis for future immunotherapy.

## **Optimization of FGL2 Monoclonal Antibody Production from Hybridoma Cells**

*Mitchell Harding, Cheryl Bodnar, Justin Manuel, Gary Levy*

An estimated 270-300 million people worldwide are infected with hepatitis C, a disease that can become life threatening. Plasma FGL2 levels, which have been shown to be a predictive indicator of the outcome of this infection, are important parameters to track in patients suffering with this disease. The Levy lab has developed an enzyme linked immunosorbent assay (ELISA) that allows clinicians to test patient plasma levels for FGL2. The limiting factor in the use of this assay is the availability of the capture antibody, a human FGL2 MAb. This study focused on the enhancement of the existing production of monoclonal antibody of this cell line through cell culture media, and temperature variations. Three types of serum free media (CD Hybridoma, Hybridoma SFM, and PFHM2- Protein Free Hybridoma Medium) were compared to each other and to a control serum based medium (Dulbecco's Modification Eagle's Media (DMEM) with 10% Fetal Bovine Serum (FBS)). CD-Hybridoma was shown to have better monoclonal antibody production than the other three cell culture media. A long term comparison experiment with this medium and DMEM with 10% FBS was then conducted, looking at monoclonal antibody production over the course of 5 cell passages. CD-Hybridoma demonstrated sustained elevated monoclonal antibody production compared to the control serum medium in this experiment as well. Further optimization of antibody production will be attempted using temperature variation with cells growing in this serum free medium. Literature review suggests that monoclonal antibody production may be optimized by temperature shifting, either from 37°C to 32°C on day 3 of the cell cycle, or from 32° to 37°C on day 2 of the cell cycle.

### **Optimization of sfgl2 production from concanavalin A-stimulated splenocytes**

*Michael Law, Jihong Wang, Jianhua Zhang, Justin Manuel, Itay Shalev, Agata Bartczak, Cheryl Bodnar, Andre C. Siegel, Gary A. Levy*

Fibrinogen-like protein 2 (fgl2), also known as fibroleukin, is a member of the fibrinogen-related protein superfamily. The membrane bound form of fgl2 (mfgl2) exhibits prothrombinase activity and is found on the surface of macrophages and endothelial cells. The secreted form of fgl2 (sfgl2) exhibits immunoregulatory activity and is secreted from CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Sfgl2 has been found to inhibit T cell proliferation and DC maturation, thus exhibiting immunosuppressive properties. The mechanism for the production of the two forms of fgl2 as well as their structural relationship has yet to be fully elucidated. It is imperative that sfgl2 production be optimized before subsequent purification and sequencing can occur. The *in vitro* production of sfgl2 from wild-type and fgl2<sup>+/-</sup> C57B/6 derived splenocytes showed dependence on Con A and IFN- $\gamma$  dosage, cell density, media, and culture time. The data suggests that a narrow range of conditions is responsible for optimal sfgl2 production. Once the immunosuppressive activity of sfgl2 is fully understood, its potential as a therapeutic agent in treating autoimmune disorders and transplant rejection can be pursued.

### **Do Palliative Care Instruments Predict Heart Failure Severity When Compared to Standardized Heart Failure Instruments?**

*Matthew Timmons, RN, BScN, Jane MacIver, RN, MSc, Dr. Heather J. Ross, MD, MHSc,*

**Background:** Heart failure (HF) is a public health problem. As HF patients approach death, many experience a decreased quality of life. It is difficult to establish prognosis in patients with HF because of their unpredictable disease course trajectory. As such, there is a reluctance to incorporate palliative care into the management of HF patients. The Palliative Performance Scale (PPS), and the Edmonton Symptom Assessment Score (ESAS), which are used to determine disease severity and the allocation of palliative care services in oncology patients, have not been studied in the HF population. Three instruments that are used in HF to determine disease severity and prognosis are the Minnesota Living with Heart Failure Questionnaire (MLHFQ), the Visual Analogue Scale (VAS), and the Seattle Heart Failure Model (SHFM). These instruments are not used for the purposes of allocating palliative care resources to patients with HF. The purpose of this study is to determine if the PPS and ESAS scores correlate with scores on the standardized heart failure questionnaires, specifically, the MLHFQ, the VAS, and the SHFM.

**Methods:** We administered the PPS, ESAS, MLHFQ, VAS, and SHFM to 27 patients with HF. Descriptive statistics were calculated for each instrument. Associations were measured using Pearson's Correlation Coefficient.

**Results:** A strong positive correlation of 0.819 was seen between the PPS and the VAS. Similarly, a strong negative correlation of -0.805 was found between the PPS and the MLHFQ. The strong negative correlation seen suggests that low scores on the PPS correlate very well with high scores on the MLHFQ.

**Conclusions:** Scores on the palliative care instruments correlate with quality of life heart failure questionnaires scores. Hence palliative care instruments can be used to determine allocation of palliative care services in patients with heart failure.

## **Bone Marrow-Derived Lung Progenitor Populations as Biomarkers for Human End-Stage Lung Disease Severity**

*Kalvin C Lung, Sarah E Gilpin, MSc<sup>1</sup>, MSc<sup>1</sup> and Thomas K Waddell, MD, PhD<sup>1</sup>. <sup>1</sup>University Health Network, Toronto, Canada.*

**BACKGROUND:** Two bone marrow–derived lung progenitor cell populations, Clara Cell Secretory Protein (CCSP)-expressing cells and circulating fibrocytes, contribute to both lung disease pathology and repair.

**RATIONALE:** Clinical parameters are currently used to evaluate the severity of respiratory diseases such as IPF, COPD, and CF. However, consensus is lacking for the most predictive parameters, and results from spirometry and exercise tests may be subjective. We hypothesize that these BM-derived cells may serve as a biological marker to provide an alternative predictor of clinical disease status and disease activity.

**METHODS:** Bone marrow and peripheral samples collected at the time of lung transplantation were analyzed by flow cytometry for CCSP<sup>+</sup> cells and CD45/Collagen1<sup>+</sup> fibrocytes (n=149). Samples from lung donors were analyzed as non-diseased controls (n=26). Patient disease status was defined using clinical parameters published in current literature. Statistical analysis was used to identify correlations between disease severity and cell population data.

**RESULTS:** CCSP<sup>+</sup> cells were increased in peripheral blood of CF patients, and significantly decreased in BOS patients. Circulating fibrocytes were significantly increased in both BOS and pulmonary fibrosis patients. No difference was found between genders or age for both cell types. The ratio of CCSP<sup>+</sup> cells to fibrocytes can significantly distinguish fibrotic (IPF/BOS) from other (CF/COPD/PH) lung diseases.

**CONCLUSION:** This study provides evidence that specific bone marrow-derived cells can help distinguish different end-stage lung diseases.

## **An Orthotopic Mouse Model of Hepatocellular Carcinoma**

*M. Lu, K., Chen, S., Ahmed, M., Böhnert, A., Ali, A., Ghanekar.*

A useful method in understanding Hepatocellular Carcinoma (HCC) involves using mice as hosts to grow tumours from cancerous cell lines. We sought to develop a model to facilitate the growth of HCC in the mouse liver. Previous experiments in our lab demonstrated that direct injection of liver cancer cell lines into the spleen or liver rarely results in significant tumor formation. We hypothesized that tumor formation would be promoted by performing a partial hepatectomy in the mouse prior to cell injection, as the regenerating liver releases many mitogens and growth factors which foster cell growth, including potential tumour formation. We first sought to evaluate this hypothesis in a syngeneic model by injecting BALB/c mice with BALB/c-derived Tib-75 mouse liver cancer cells grown *in vitro*. 2 groups of BALB/c mice were used for Tib-75 injection into the liver (n=6) and the spleen (n=6.) Adherent Tib-75 cells were isolated into aliquots of  $1.5 \times 10^6$  cells in 50 $\mu$ L of PBS. 3/4 Partial Hepatectomy (PH) was performed on BALB/c mice. Tib-75 aliquots were then injected into the liver or spleen and allowed to grow for 6 weeks. The results demonstrated significant tumor formation in the liver following liver or splenic injection. Thus, the regeneration of the liver contributed to the development of tumours. This model can be utilized to investigate the relationship between liver regeneration and tumorigenesis, and can also be applied to a xenogeneic model to facilitate the growth of human HCC in the livers of immunodeficient mouse strains.

## **The effect of immunosuppressant drug therapies targeted against human hepatocellular carcinoma cell lines**

*A., Ali, M., Ma, S., Ahmed, K., Chen, M. lu, A., Ghanekar.*

Human Hepatocellular carcinoma (HCC) is one of the ten most frequent carcinomas worldwide. The most effective treatment for HCC is liver transplantation; however, long-term immunosuppressant treatment is required after the surgery. Immunosuppressant drugs administered after the surgery include: Tacrolimus, Cyclosporine and Rapamycin. A common phenomenon is the reoccurrence of tumors after transplantation. This study is aimed at investigating the effect of immunosuppressant drug therapies on the proliferation of a tumorigenic human HCC cell line, Huh 7, as well as the effect on isolated stem cell populations within the cell line.

Huh 7 cells were retrieved from cryo preservation and a cell culture system was established. A cell density of 5000cells/well was seeded on a 96 well plate and treated with Tacrolimus 20ng/mL, Cyclosporine 100ng/mL, Rapamycin 20ng/mL and an untreated control group. After 7 days, a MTT assay was conducted to measure the cell proliferation. The bulk huh7 cells were sorted using miltenyi MACS, magnetic cell sorting to isolate populations exhibiting two stem cell surface markers: CD133 and EpCAM. The isolated cells were also treated with the three drugs.

Results show that Rapamycin works effectively as an anti-proliferative drug and the isolated stem cell populations appear to behave differently when treated with the drugs. Some drugs are more effective than others at reducing cell proliferation of tumorigenic cell lines. If stem cell populations responsible for tumor formation are specifically targeted with these drug therapies, these treatments can more effectively function to minimize the reoccurrence of tumors after transplantation.

## **The Role of Chemokines and Chemokine Receptors in Avian B-cell Development**

*A. Borjian, A. Ling, D. Tahamzadeh, and M. Ratcliffe*

B-cell development is a tightly regulated process to produce a repertoire of functional B-cells. As opposed to mammalian system, the Bursa of Fabricius is the primary lymphoid organ in the avian system. During the B-cell development, embryonic B lineage precursor cells and large basophilic dendritic cells colonize the mesenchyme of the Bursa. B-cell precursors then undergo gene re-arrangement to express surface immunoglobulin (slg) after which they follow the dendritic-like cells into the developing bursal follicle. At the time of hatch, the bursal follicle is presented with antigens transported from the bursal lumen which diversifies the Ig receptors through gene conversion. The bursal follicle then differentiates into two medullary and cortical regions. The B-cells within the follicular cortex have a higher rate of cell division and are smaller in size compared to B-cells within the medullary region. Considering these district migratory events involved in the B-cell development within the bursa, we hypothesize that there are chemokine secretions and chemokine receptor expression on the B-cells controlling these processes. To understand what chemokines and chemokine receptors are involved we investigated their expression in large and small B-cells, as an approximation for the medulla and cortex, and non B-cells, representing the microenvironment of the bursa. The expression of chemokine and chemokine receptors were analyzed using Quantitative Real-Time PCR. The results illustrate that there is a high expression of CXCR4, CXCR5, and CXCR7 in small B-cells relative to the large B-cells. In addition, the chemokine ligands CXCL8b, CXCL12, and CXCL13b are expressed at high levels within the microenvironment of the bursa. These results suggest that there is a possibility of involvement of these chemokines and chemokine receptors in the avian B-cell development.

## Characterization of the Role of FGL2 in the Generation of a B-cell Response to Lymphocytic Choriomeningitis Virus (LCMV) WE

Natalie Yavorska, Ramzi Khattar, Jianhua Zhang, Justin Manuel, Cheryl Bodnar and Gary A. Levy.

**Background and Aims:** FGL2, a member of the fibrinogen-like protein super family, is a novel Treg effector molecule that mediates its activity by binding to the inhibitory FCγRIIB receptor expressed on antigen presenting cells. FGL2 has been previously shown to play a key role in regulating both innate and adaptive immunity. Binding of FGL2 to FCγRIIB receptor induces the apoptosis of B cells. The aim of this study is to assess the role of FGL2 in regulating B cell responses to Lymphocytic Choriomeningitis Virus (LCMV)-induced hepatitis infection.

**Methods:** Both wild-type and *fgl2* knockout mice were infected with  $2 \times 10^6$  PFU of LCMV WE and subsequently bled into heparinized tubes on a weekly basis from infection. Plasma was obtained from the collected samples by centrifugation. Plasma titres of LCMV specific antibodies were measured by ELISA. The levels of CD138<sup>+</sup>CD19<sup>Low</sup>CD45R<sup>Low</sup> plasma cells were assessed by flow cytometry in the lymph node and spleen. Neutralizing antibody was assessed through the use of an LCMV focus forming assay and the ability for plasma to neutralize plaque formation.

**Results:** LCMV-specific antibodies were higher in *fgl2* knockout mice than in wild type mice throughout the infection. Plasma cell numbers were also elevated in spleens of *fgl2* knockout mice before infection and eight days post infection when compared to the wild-types. This trend was not observed in lymph node plasma cell counts. Preliminary data testing for neutralizing antibody suggests that the *fgl2* knockout does indeed have increased levels of neutralizing antibody toward LCMV when compared to wild-type mice, however more optimization of the assay is required to confirm these observations.

**Conclusion:** These preliminary results demonstrate a novel role for FGL2 in regulating B cell responses to LCMV. Further studies of the immunoregulatory properties of FGL2 will be critical in furthering our understanding of hepatitis and lead to the development of therapeutics to treat patients suffering from this debilitating disease.

## Natural Killer Cell Characterization in a Tolerized Allograft Model

A. Pargaru, P. Urbanellis, W. He, J. Wang, L. Morikawa, I. Shalev, A. Bartczak, R. Khattar, and G. Levy.

Reducing immunosuppressive therapy and inducing allograft tolerance remain the main therapeutic goals in transplant immunology. Recently, our lab demonstrated long-term graft survival without immunosuppression in an established, fully major histocompatibility complex (MHC) mismatched, murine heterotopic heart transplant model. In this model, we noticed elevated levels of *klrh1* expression in mice that had tolerated the graft. KLRH1 is an inhibitory receptor found on both Natural Killer (NK) cells and NK-T cells. We hypothesize that NK cells mediate rejection, while the increased expression of KLRH1 contributes to graft acceptance. C3H mouse recipients of fully MHC mismatched hearts from BALB/c mice were treated with Rapamycin for 16 days. The heart graft, lymph nodes and spleen were harvested 30 days after transplantation for immunohistochemistry and flow cytometry. Pure populations of NK cells were used in chromium release assay and gene profiling. Preliminary flow cytometry results showed decreased NK cell count in tolerant mice. Cytolytic activity towards donor and 3<sup>rd</sup> party antigens was higher in NK cells from tolerant mice than graft rejecting mice. Current data suggests that NK cells do not mediate graft acceptance. Rapamycin treatment may decrease in NK cell numbers, contributing to graft tolerance. Further data analysis and pending *klrh1* gene expression profiling may explain the observed graft acceptance.

**Interferon gamma signaling in *lpr* double negative T cells is required for their ability to kill syngeneic alloreactive CD4<sup>+</sup> T cells.**

*Caroline Jeon, Stephen C. Juvet, Betty Joe, and Li Zhang*

Regulatory T cells (Treg) are an attractive option for modulating allogeneic immune responses such as graft-versus-host disease (GVHD) and allograft rejection. These processes are the result of T cells recognizing the target tissue as foreign. We and others have demonstrated that  $\alpha\beta$  T cell receptor-expressing CD4<sup>-</sup>CD8<sup>-</sup> non-NK double negative (DN) Tregs from mice and humans can inhibit alloimmune (host-versus-graft and graft-versus-host) responses mediated by CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Mice homozygous for Fas deficiency (*lpr* mice) accumulate DN T cells that have been shown to be regulatory *in vitro* and *in vivo*. DN Tregs from *lpr* mice secrete interferon gamma (IFN $\gamma$ ) and must do so in order to inhibit GVHD. To better characterize the role of IFN $\gamma$  in DN Treg function we have bred wildtype *lpr*, IFN $\gamma$ -deficient (IFN $\gamma$ <sup>-/-</sup>) *lpr* and IFN $\gamma$  receptor-deficient (IFN $\gamma$ R<sup>-/-</sup>) *lpr* mice, all on the B6 (H-2<sup>b</sup>) background. We hypothesized that IFN $\gamma$  is required for DN Tregs to kill syngeneic alloreactive T cells. To test this hypothesis, *lpr*, *lpr*.IFN $\gamma$ <sup>-/-</sup> and *lpr*.IFN $\gamma$ R<sup>-/-</sup> DN Tregs were purified and activated *in vitro* with irradiated allogeneic CB6F1 (H-2<sup>b/d</sup>) splenocytes and then tested as cytotoxic effector cells in an 18h JAM assay against <sup>3</sup>H-thymidine labelled B6 CD4<sup>+</sup> targets activated in a similar manner. We found that while IFN $\gamma$ <sup>-/-</sup> DN Tregs were less efficient at killing syngeneic CD4<sup>+</sup> targets in comparison with wildtype DN Tregs (Two-way ANOVA p=0.009), IFN $\gamma$ R<sup>-/-</sup> DN Tregs were entirely unable to kill syngeneic CD4<sup>+</sup> targets (Two-way ANOVA p<0.0001). These data indicate that IFN $\gamma$  signalling in DN Tregs is absolutely required for DN T cell cytotoxic function, and suggest that DN Tregs may use IFN $\gamma$  in autocrine fashion to act as regulatory cells during allogeneic immune responses. Studies are currently underway to determine how IFN $\gamma$  exerts its pro-regulatory effects on *lpr* DN Tregs.