INTERNATIONAL SOCIETY FOR HEART AND LUNG TRANSPLANTATION (ISHLT)

BASIC SCIENCE & TRANSLATIONAL RESEARCH CORE COMPETENCY CURRICULUM (ISHLT BSTR CCC)

SECOND EDITION

THE EDUCATIONAL WORKFORCE OF THE
ISHLT BASIC SCIENCE & TRANSLATIONAL RESEARCH COUNCIL

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ISHLT BSTR CCC: List of Contents

I. Introduction and Overall Goals

The purpose of this compendium is to provide a Core Competency Curriculum in Basic Science and Translational Research (BSTR) as it relates to heart and lung failure and transplantation. This curriculum does not replace a textbook, but intends to provide an outline of essential topics in the field of BSTR as well as references and hyperlinks that should be considered for individual study to develop competencies in various aspects of BSTR. The ISHLT BSTR Academy will focus on core competencies in Basic Science and Translational Research and will fill gaps in practice by assisting clinicians in improving their understanding of the scientific background behind clinical practice, updating basic and translational researchers on recent discoveries, encouraging interaction between basic/translational researchers and clinicians, and stimulating discussion about common basic topics in the fields of heart versus lung failure and transplantation. The Educational Workforce of the ISHLT BSTR Council recognizes the role of BSTR in every discipline of the ISHLT and acknowledges the challenges of accommodating all disciplines in this BSTR Core Competency Curriculum. This compendium and the ISHLT BSTR Academy will therefore focus on basic concepts in immunology and molecular biology related to heart and lung transplantation. Future developments and addendums of this Curriculum may integrate other disciplines. We welcome constructive feedback to further develop the scope and accuracy.

Educational Goals

The educational goals of this activity are to provide a concise review of basic concepts in transplant-related immunology and molecular biology, to define and promote the clinical relevance of BSTR related to heart and lung transplantation, and to encourage interaction between basic scientists, translational researchers, and clinicians via a networking opportunity.

Learning Objectives

After completing this curriculum, participants will have improved competency and professional performance in their abilities to:

1. Understand basic concepts in transplant-related immunology and molecular biology;
2. Recognize key analytical techniques and models used in transplantation research;
3. Understand basic mechanisms of immunosuppression;
4. Recognize key contributions from basic research in transplantation that improved clinical outcomes in heart and lung transplantation;
5. Recognize how clinical questions may inspire basic research;
6. Enable effective communication between research scientists and clinicians and health care professionals.

II. BASIC IMMUNOLOGY

Learning Objectives for Basic Immunology:

1) **Understand the principles of inflammation and the role of the complement system;**
2) **Describe the cells of the innate and adaptive immune system and their mode of action;**
3) **Distinguish between properties of innate immunity and adaptive immunity;**
4) **Understand how immune cells recognize antigens;**
5) **Understand the processes of cell-cell interaction and activation;**
6) **Describe the immunoregulatory mechanisms for the control of (self-)reactive cells;**
7) **Understand the concept of immunologic memory.**

1. **Innate immunity**
   - A. Inflammation
   - B. Complement system
   - C. Innate immunity cells
     - i. Macrophages
     - ii. Dendritic cells
     - iii. Natural killer cells
     - iv. Neutrophils
     - v. Mast cells
   - D. Toll-like receptors (TLR)
     - i. Diversity
     - ii. Ligands
     - iii. Signalling
2. **Adaptive immunity**
   - A. Adaptive immunity cells
     - i. T cells
     - ii. B cells
     - iii. NKT cells
   - B. Cell-cell interaction
     - i. Co-stimulation
     - ii. Cytokines/chemokines
   - C. Antigen recognition
     - i. T cells
       - a. T cell receptor
       - b. Major histocompatibility complex (MHC)

ii. B cells
    a. B cell receptor
    b. Antibodies
iii. NKT cell
    a. CD1d molecule

D. Cell activation/signalling

E. Cell regulation

i. Anergy
ii. Deletion
iii. Ignorance
iv. Active suppression

F. Naïve versus memory

G. Cell death

i. Programmed cell death/apoptosis
ii. Necrosis

Selected References:

Textbooks

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- Fink SL et al. **Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells**. Infect Immun, 2005. 73: 1907.

Selected Hyperlinks:

Posters

- **Innate immunity**: http://www.nature.com/nri/posters/innate/nri0804_ii_poster.pdf
- **Dendritic cells**: http://www.nature.com/nri/posters/dendriticcells/nri1107_dendriticcells_poster.pdf
- **NK cells**: http://www.nature.com/nri/posters/nkcells/nri1012_nkcells_poster.pdf
- **T cell subsets**: http://www.nature.com/nri/posters/tcellsubsets/nri1009_tcellsubsets_poster.pdf
- **B cell subsets:** http://www.docstoc.com/docs/113396676/b-cell-poster
- **Antigen processing**: http://www.nature.com/nri/posters/antigenprocessing/nri0905_antigen_poster.pdf

Videos

- **Complement system**: http://www.youtube.com/watch?v=vbWYz9XDtLw
- **Innate pathogen recognition:** http://www.youtube.com/watch?v=gRKHeDzfh0Y&list=PL7D18C93964A61F67
- **MHC class I processing**: http://www.youtube.com/watch?v=vrFMWyJwGxw&list=PL7D18C93964A61F67
- **MHC class II processing**: http://www.youtube.com/watch?v=_8JMVq7HF2Y&list=PL7D18C93964A61F67
- **TCR-APC interaction**: http://www.youtube.com/watch?v=Xt_y7f6KivI&list=PL7D18C93964A61F67
- **Immunological synapse**: http://www.youtube.com/watch?v=R4zuWO5krAw&list=PL7D18C93964A61F67
- **Monoclonal and polyclonal antibodies:** http://www.youtube.com/watch?v=I-QSIwyUIy8
- **Necrosis versus apoptosis**: http://www.youtube.com/watch?v=4wPlw_Bdz7Q

III. TRANSPLANT IMMUNOBIOLOGY

Learning Objectives for Transplant Immunology:

1) **Define the mechanisms behind ischemia reperfusion injury;**
2) **Understand the concept of allorecognition;**
3) **Define the mechanistic differences and interactions between cellular and humoral, acute and chronic, rejection;**
4) **Recognize the basis for current controversies in the diagnosis and treatment of antibody-mediated rejection;**
5) **Describe major mechanisms of immunologic regulation and tolerance in transplantation.**

1. **Ischemia reperfusion injury**
   - A. Mechanisms
2. **Immune reactivity to alloantigens**
   - A. Alloantigens
     - i. ABO blood group system
     - ii. HLA antigens and HLA antigen nomenclature
     - iii. Non-HLA antigens
   - B. Mechanisms of allorecognition
     - i. Direct
     - ii. Indirect
     - iii. Semi-direct
     - iv. Role of intra-graft T cell activation and tertiary lymphoid organs
   - C. Antibody-mediated versus cellular rejection
     - i. Critical concepts and controversies in detection and treatment of antibody-mediated and cellular rejection
     - ii. Mechanisms of T cell-based rejection
     - iii. Mechanisms of B cell-based / antibody-mediated rejection
     - iv. Anti-HLA antibodies in rejection
       - ii. HLA antibody nomenclature
       - iii. HLA mismatch
       - iv. Eplet/epitope mismatch
     - i. Auto-antibodies in rejection
   - B. Type of allograft rejection

i. Hyperacute rejection
ii. Acute rejection
iii. Chronic rejection
    a. Bronchiolitis obliterans syndrome (BOS) in lung transplantation and epithelial injury
    b. Chronic allograft vasculopathy (CAV) in heart transplantation and endothelial injury
    c. Fibrosis pathways

3. Immune regulation of alloantigen response

A. Mechanisms of immune regulation

i. Deletion
ii. Active suppression by regulatory cells
    a. Regulatory T cells
    b. Regulatory B cells
    c. Regulatory macrophages
    d. Tolerogenic dendritic cells
    e. Myeloid-derived suppressor cells
    f. Stem cells
    g. NK cells

B. Definition of tolerance

C. Definition and possible mechanisms of accommodation

4. Immunity against infectious agents

A. Host-defense during post-transplant infections

i. Colonization versus infection
ii. Bacterial (e.g. Pseudomonas)
iii. Mycobacterial
iv. Fungal (e.g. Aspergillus)
v. Viral
    a. Latent (e.g. CMV, HSV)
    b. Community acquired respiratory viruses

B. Effect of infections on alloreactivity, rejection, and outcomes

5. Microbiome and its interaction with the immune system

A. Bacterial microbiome

B. Virome

C. Fungome

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Selected Hyperlinks:

Posters

- **Regulatory T cells**: http://www.nature.com/nri/posters/tregcells/index.html
- **Myeloid-derived suppressor cells**: http://www.nature.com/nri/posters/mdscs/nri1005_mdscs_poster.pdf

Videos

- **Transplant immunology and rejection: Fundamentals.** https://www.youtube.com/watch?v=F9UWVSZ0E4g
- **Memory cells and rejection: Improving transplant results.** By Dr. Alan Kirk at Emory**.** https://www.youtube.com/watch?v=Ut5Q4XnZtnE

IV. IMMUNOSUPPRESSION AND IMMUNOMODULATION

Learning Objectives for Immunosuppression:

1) **Review the history of immunosuppression in heart and lung transplantation;**
2) **Recognize major categories of immunosuppressive agents used in heart and lung transplantation;**
3) **Describe the mode of action of immunosuppressive agents;**
4) **Discuss novel targets in immunosuppression and novel pathways and drugs in the pipeline;**
5) **Understand the principle of cell-based strategies to induce transplant tolerance.**

1. **Overview of immunosuppressive agents and their mode of action**
   - A. Current immunosuppressive agents
     - i. Calcineurin inhibitors
     - ii. Cell-cycle inhibitors
     - iii. Target-of-Rapamycin inhibitors
     - iv. Steroid agents
     - v. Monoclonal and polyclonal antibodies
     - vi. Other agents
   - B. Novel immunosuppressive agents (in experimental use only)
2. **Systemic mechanical immunosuppression**
   - A. Total body irradiation
   - B. Total lymphoid irradiation
   - C. Plasmapheresis, immunoabsorption and photopheresis
   - D. Other
     - i. Splenectomy
     - ii. Thymectomy
     - iii. Non-mainstream techniques
3. **Cellular therapy**
   - A. T cells
     - i. Regulatory T cells
     - ii. Car-T cells
   - B. Regulatory B cells
   - C. Regulatory macrophages
   - D. Tolerogenic dendritic cells
   - E. Myeloid-derived suppressor cells

F. Stem cells and progenitor cells

   i. Multipotent stem cells
   ii. Pluripotent stem cells
      a. Embryonic stem cells
      b. Induced pluripotent stem (iPS) cells
   iii. Progenitor cells

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Journal Articles

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- Budniatzky I et al. **Concise Review: Reprogramming Strategies for Cardiovascular Regenerative Medicine: From Induced Pluripotent Stem Cells to Direct Reprogramming**. Stem Cells Transl Med, 2014.

Selected Hyperlinks:

- **Generic Drug Immunosuppression in Thoracic Transplantation: An ISHLT Educational Advisory**: https://www.ishlt.org/ContentDocuments/JHLT_July2009_Generic_Concensus_Statement.pdf

V. Research Models, Assays, and Technologies

Learning Objectives for Research Models and Clinical Assays:

1) **Review the different pre-clinical animal models in transplantation research;**
2) **Understand the role of animal models to answer specific transplant-related questions;**
3) **Discuss the advantages and disadvantages of pre-clinical animal models;**
4) **Describe major sources of human samples for translational research;**
5) **Understand key lab techniques to measure innate and adaptive immune responses and to monitor transplant recipients;**
6) **Recognize imaging techniques used to analyze organ function, cell survival and lymphocyte trafficking.**

1. **Pre-clinical models**
   - A. Animal models – the pros and cons
     - i. Murine
     - ii. Pig
     - iii. Primate
     - iv. Ex-vivo conditioned organs
   - B. Diversity in models
     - i. Transgenic models
       - a. Green Fluorescence Protein (GFP) models
       - b. Firefly luceriferase (fluc) models
       - c. Other
     - ii. Knock-out/in models
     - iii. Humanized models
   - D. Key models of rejection in lung and heart Tx
2. **Samples for translational and clinical science**
   - A. Human samples
     - i. Blood, serum, plasma
     - ii. Lung and heart tissue (biopsies)
     - iii. Bronchoalveolar lavage (BAL): supernatant vs. cells
     - iv. Explanted allografts (autopsy, retransplant)
     - v. Explanted thymuses
     - vi. Donor or recipient lymph nodes
   - B. Sample processing to answer specific research questions
     - i. Centrifugation
     - ii. Filtration

iii. Preservation agents
iv. Cryopreservation
v. Biobanking

3. Key analytical techniques

A. Protein analysis

i. Flow cytometry
   a. Cell characterization
   b. Antibody titres
   c. Cell surface proteins
   d. Intracellular proteins
ii. Multiplex
iii. Luminex
iv. ELISA
v. ELIspot
vi. Western Blot
vii. Immunohistochemistry and immunofluorescence
viii. Proteomics

B. DNA analysis

i. Genomic PCR
ii. DNA degradation

C. Gene expression analysis / transcriptomics

i. Real-Time PCR
ii. Gene expression arrays (microarrays)
iii. Single-cell RNA sequencing
iv. Epigenetics

D. MicroRNA analysis

E. Cellular Functional Assays

i. MLR
ii. CTL
iii. Other

F. Histopathology

i. Stains: Hematoxylin-Eosin, Masson Trichrome, PAS, EVG, others
ii. ISHLT grading or rejection

iii. Immunestaining/immunofluorescence

G. Imaging

i. Echo
ii. Molecular Imaging
iii. Bioluminescence imaging (BLI)
iv. Coronary imaging
v. Chest imaging
vi. microCT

H. Novel technologies

4. Novel technologies for improved diagnostics

A. Biomarkers of rejection

B. Molecular microscope

C. Blood gene expression signatures

D. Graft-derived gene expression signatures

E. MicroRNA analysis

F. Circulating donor cell-free DNA

G. Intra-graft immune cell analyses

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Web pages:

- **The use of animal models to study genetic disease:** http://www.nature.com/scitable/topicpage/the-use-of-animal-models-in-studying-855
- **Transgenic mouse models of human disease:** http://labs.medicine.ucsf.edu/chrislau/GFP.html
- **What is PCR:** http://www.genome.gov/10000207
- **Guidelines for optimizing PCR – DNA analysis:** http://www.agctsequencing.com/Articles/PCROptimize1.pdf
- **Real-time PCR tutorial**: http://pathmicro.med.sc.edu/pcr/realtime-home.htm
- **ELISA:** http://www.bio.davidson.edu/genomics/method/ELISA.html
- **Western blotting:** http://www.piercenet.com/method/overview-western-blotting

Videos:

- **DNA microarrays:** http://www.youtube.com/watch?v=VNsThMNjKhM
- **DNA microarrays:** http://www.youtube.com/watch?v=9U-9mlOzoZ8
- **Epigenetics overview:** http://www.youtube.com/watch?v=Tj_6DcUTRnM
- **Development of Obliterative Bronchiolitis in a Murine Model of Orthotopic Lung Transplantation:** http://www.jove.com/video/3947/development-obliterative-bronchiolitis-murine-model-orthotopic-lung