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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(V1.1 NOVEMBER 2018)
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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

Journal Articles
• Chen GY et al. Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol, 2012. 10: 826.

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=vrFMyJwGxw&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=R4zuWOSkrAw&list=PL7D18C93964A61F67
• Monoclonal and polyclonal antibodies: http://www.youtube.com/watch?v=I-QSlyyUly8
• 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 allore cognition;
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 allore cognition
      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**
**Selected References:**

**Journal Articles**

• Dorling A. Transplant accommodation – are the lessons learned from xenotransplantation pertinent for clinical allotransplantation? Am J Transplant. 2012. 12: 545.
• Calabrese DR, Lanier LL, Greenland JR. **Natural killer cells in lung transplantation**, Thorax. 2018

**Selected Hyperlinks:**

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

**Videos**
- Transplant immunology and rejection: Fundamentals. [https://www.youtube.com/watch?v=F9UWVSZ0E4g](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](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
Selected References:
Journal Articles


Selected Hyperlinks:

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 luciferase (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. Immunostaining/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

Selected References:

- Bribriesco AC et al. Experimental models of lung transplantation. Front Biosci, 2013. 5: 266.
Selected Hyperlinks:

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
- Real-time PCR tutorial: http://pathmicro.med.sc.edu/pcr/realtime-home.htm
- 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-9mIOzoZ8
- Epigenetics overview: http://www.youtube.com/watch?v=Tj_6DcUTRnM